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From the Department of Physiology, University of Göteborg, Göteborg, Sweden

The Comparative Effects of Angiotensin and Noradrenaline on Consecutive Vascular Sections

By

BJÖRN FOLKOW, BÖRJE JOHANSSON and STEFAN MELLANDER

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Abstract

FOLKOW, B., B. JOHANSSON and S. MELLANDER. *The comparative effects of angiotensin and noradrenaline on consecutive vascular sections.* Acta physiol. scand. 1961. *53.* 99—104. — The action of l-noradrenaline and angiotensin on the resistance and the capacitance vessels in a skin-muscle region of the cat was studied by means of a technique recently described by MELLANDER (1960). Intravenous and close arterial infusions of the two substances showed that the constrictor effect of angiotensin on the resistance vessels is greater than that of corresponding amounts of noradrenaline. In the capacitance section, on the other hand, the constrictor responses to noradrenaline by far exceed those to angiotensin. On the basis of these results it is suggested that noradrenaline 'receptors' are to be found on the arterial as well as the venous side of the circulation, while angiotensin 'receptors' are essentially confined to the smooth muscles of the precapillary vascular region.

Among the various vasoactive substances formed in the organism angiotensin is of special interest owing to its potency and its possible role in certain forms of experimental and clinical hypertension. During the last few years the chemical structure of different angiotensin compounds has been established and the preparation of synthetic analogues has greatly facilitated investigations on the pharmacology of these substances.

The present stage of knowledge concerning the cardiovascular action of angiotensin has recently been reviewed (SCHACHTER 1960, p. 95) and will here

only be briefly summarized. Angiotensin II, now considered to be the final link in the renin-angiotensin system, appears to constrict powerfully the resistance vessels in all systemic vascular regions with possible exception of the coronary vessels. The circulatory effects of angiotensin on the different 'parallel-coupled' vascular circuits supplying the various tissues, are thus fairly well-known as regards their 'resistance vessels'. However, the action of vasoactive agents should also be considered from another point of view. Each regional vascular circuit is made up of a number of functionally differentiated 'series-coupled' sections, which can be called 'Windkessel vessels', 'resistance vessels' with a precapillary and a postcapillary section, 'sphincter vessels', 'exchange vessels', 'capacitance vessels' and, in some tissues 'shunt vessels'. (For details see *e. g.* FOLKOW 1959 or MELLANDER 1960.) Not only reactions of the resistance vessels, as a whole are here of importance but also the relation of constriction, or dilatation, between the precapillary and postcapillary resistance vascular sections, since this ratio will affect mean hydrostatic pressure in the capillaries and hence the filtration exchange across their walls. Further, the effects on the tone of the capacitance vessels are also highly important as they will markedly affect the venous return of blood to the heart.—As the phasic reactions of these functionally differentiated consecutive vascular sections can now be studied simultaneously in a skin-muscle region in the cat, by means of a technique recently described by MELLANDER (1960), it was considered of interest to utilize this method for a comparative study of the action of angiotensin and noradrenaline on resistance and capacitance vessels. — The present results have previously been briefly outlined (FOLKOW *et al.* 1960).

Method

The present investigation was performed on 15 cats, anaesthetized with chloralose (50 mg/kg) and urethane (100 mg/kg) intravenously. In a few cases dial (Ciba, 30—40 mg/kg) or urethane alone (1,000—1,500 mg/kg) was used, but the different anaesthetics did not in principle affect the results. As indicated above, a simultaneous recording of phasic reactions in consecutive vascular sections is possible by means of the method used in these experiments. This method has been described and tested in detail by MELLANDER (1960) and is also in principle described in another paper in the present volume (FOLKOW, MELLANDER and ÖBERG 1961). It allows an analysis of the extent of the reactions of the pre- and postcapillary resistance vessels separately, as well as the capacitance vessels. — As the direct action of the vasoactive agents upon the vessels of the hind quarters was to be analysed, the lumbar sympathetic chains were generally cut in the course of the experiment, in order to record the pharmacological effects on the vascular bed *per se*, when it is deprived of all reflexogenic changes of vasoconstrictor tone. Disturbances from endogenous adrenomedullary hormones were prevented by extirpation of the right suprarenal gland and denervation of the left one.

Angiotensin II (Ciba) and l-noradrenaline were given as intra-venous and close arterial injections and infusions, by way of one of the brachial veins and a suitable lumbar branch of the aorta, respectively.

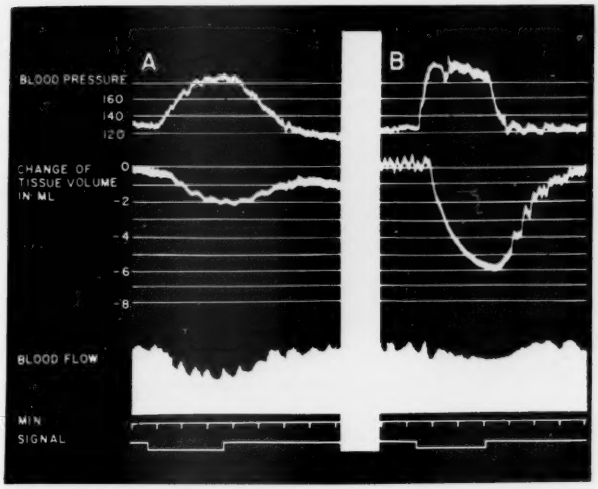

Fig. 1. Cat, chloralose-urethane.
The effects on blood pressure, 'blood volume' and blood flow of intra-venous infusion of angiotensin, 1.2 μg/kg/min (A) and l-noradrenaline, 1.2 μg/kg/min (B). Note the bigger increase of flow resistance and the smaller effect on 'blood volume' of angiotensin as compared with l-noradrenaline.

Results

Fig. 1 shows the vascular responses recorded in a representative experiment, where angiotensin and l-noradrenaline were given in intra-venous infusions. The upper tracing represents the arterial blood pressure, the middle one the changes in regional tissue volume and the lower one the regional blood flow, the height of the ordinates being directly proportional to the rate of blood flow.

In Fig. 1 A angiotensin was infused intra-venously at a rate of 1.2 μg/kg/min, which induced an increase in blood pressure and decrease in blood flow corresponding to a change in vascular resistance from 13 to 33 relative units. Regional tissue volume was slowly and moderately decreased. The two phases in the volume curve due to active constriction of the capacitance vessels and transcapillary influx of fluid are not so clearly distinguished here. However, a fall in mean capillary hydrostatic pressure can be assumed on the basis of the induced changes in vascular resistance and blood pressure. Thus, the total regional resistance has increased approximately 2.5 times the initial value and most of this increase should occur in the precapillary resistance section, since the venous side is on the whole very little affected, judging by the slight volume decrease. In spite of the elevated arterial perfusion pressure, this predominant precapillary vasoconstriction should induce a lowered capillary pressure, and hence at least part of the recorded volume change should be due to fluid reabsorption.

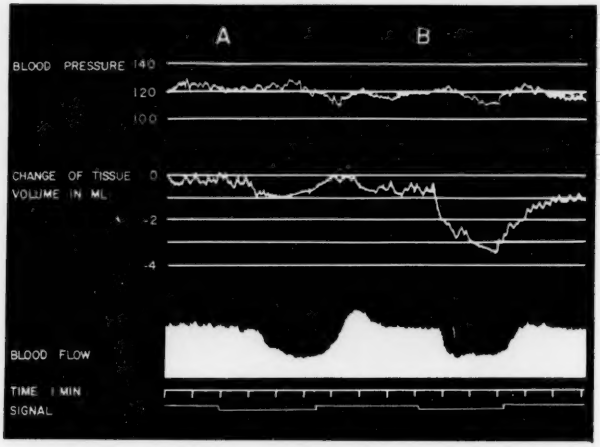

Fig. 2. Cat, chloralose-urethane.
The effects on blood pressure, 'blood volume' and blood flow of intra-arterial infusion of angiotensin, 1.4 μg/kg perfused tissue/min (A) and l-noradrenaline, 3.6 μg/kg perfused tissue/min (B). Note the bigger decrease of volume caused by l-noradrenaline, when this drug and angiotensin are given in doses that elicit approximately similar increases of flow resistance.

In Fig. 1 B l-noradrenaline, 1.2 μg/kg/min, was infused intra-venously. The elevation of arterial pressure is accompanied by a reduced regional blood flow and the vascular resistance is here increased from 13 to 22 relative units. In the plethysmographic curve the two different slope characteristics are easily distinguished: the initial, rapid decrease in volume due to active constriction of capacitance vessels and the following, continuous one, due to transcapillary absorption of extravascular fluid. In this case the contribution of these two processes to the recorded volume decrease is approximately 5 and 1.5 ml respectively. — Fig. 1 has thus revealed some differences in the action of noradrenaline and angiotensin on resistance and capacitance vessels, which will be further discussed later on.

Similar results were obtained in Fig. 2, where the two substances were administered intra-arterially. This figure illustrates first the effect of angiotensin (A), infused at a rate of 1.4 μg/kg tissue/min. As here angiotensin is not generally distributed, there is no definite change in arterial blood pressure, but the regional vascular resistance of the hind parts is gradually increased from 10 to 33 units. Tissue volume is, on the whole, very little affected, but the small initial deflection implies a minute decrease in regional blood content. This is possibly the result of a slight active constriction in the capacitance vascular section, but a mere passive reduction in the diameter of these vessels, due to intravascular pressure drop, cannot be excluded. The vascular effects at this particular infu-

sion are not stable enough to give any clear indication of significant changes in capillary fluid exchange.

In Fig. 2 B the effect of l-noradrenaline, 3.6 μg/kg tissue/min, given intra-arterially, is demonstrated. The effect of this dose on the total regional resistance is quantitatively equivalent to that of the smaller dose of angiotensin in Fig. 2 A. The volume decrease, on the other hand, is here considerable, comprising approximately 3 ml. The two phases in the volume curve are also recognized more distinctly in this recording. — Similar vascular effects were also obtained in such experiments where the doses of the two agents were lower than those here given. The effects described were easily reproduced in different experiments.

Discussion

The present experiments indicate that angiotensin induces a stronger constriction of muscular and cutaneous resistance vessels than noradrenaline, when given in equivalent doses. On the other hand the constrictor effect of noradrenaline on the capacitance vessels is much more pronounced than that of angiotensin. Thus, when the two agents were given in such amounts as to induce the same degree of constriction in the resistance vessels, the decrease in tissue volume induced by noradrenaline was up to 5 times as great as that caused by angiotensin. The difference in 'active' constriction of the capacitance vessels — mainly the veins — might be even more pronounced, since other factors can also be expected to produce a volume decrease when angiotensin is given. Thus, a constriction of mainly the precapillary resistance vessels will necessarily lower the hydrostatic capillary pressure, other factors being unchanged, and hence tend to cause an extravascular fluid absorption. Further, such a precapillary vasoconstriction implies under the prevailing circumstances a decrease in postcapillary transmural pressure, which will cause a passive-elastic recoil of the highly distensible venous side, mobilizing some of its blood content. Lastly, the constriction of the precapillary vessels will lower their blood content. Such mechanisms may to a great extent, possibly entirely, be made responsible for the slight decreases in tissue volume observed during angiotensin infusion. Since the venous side of the vascular bed constitutes the main part of the capacitance section, and the arterioles the most important resistance section, this investigation makes it likely that the smooth muscles of the veins, as well as those of the arterioles, are supplied with noradrenaline 'receptors'. On the other hand, angiotensin 'receptors' seem to be mainly, if not totally, confined to the precapillary vascular region. If angiotensin was given in such concentrations as may be expected to occur in certain types of hypertensive disease, — raising flow resistance some 50—100 %, — the effects on the venous side appeared to be negligible. If, however, the substance was given in massive doses, so as to stop blood flow almost entirely in the hind parts, rela-

tively marked volume decreases were observed, and thus it seems possible that there might be a small number of angiotensin 'receptors' also on the venous side. It should, on the other hand, be realized that under such circumstances it is no longer a matter of mimicking a reasonable patho-physiological situation in hypertensive disease, but rather a toxicologic situation. — It might be argued that angiotensin could be unevenly distributed within the vascular bed, thus reaching the arteriolar smooth muscles in higher concentrations. It should, however, be recalled that, when vasoactive substances are given by way of the blood stream, the smooth muscles of the arterioles, as well as those of the veins, are in all probability mainly reached from the tissue spaces *via* the capillary exchange, and the results show that noradrenaline must easily reach the venous side.

These results have thus revealed quantitative, and probably also qualitative, differences between the action of two pressor agents on various consecutive vascular sections, differences which would hardly be possible to discover only by means of conventional blood pressure and blood flow recordings. For a true physiological and pharmacological evaluation of the action of vasoactive drugs it seems important to apply a method that also allows a detailed analysis of the vascular responses in different 'series-coupled' sections, since each of them has a highly differentiated function in cardiovascular homeostasis.

This study has been supported by grant No. AF 61(052)-286 from the School of Aviation Medicine, USA and by grant No. H 5675, U. S. Public Health Service. — AB Hässle, Göteborg, Sweden, has generously covered the expenses for the employment of a technician. — We are greatly indebted to professor F. Gross, Ciba Ltd, Basle, Switzerland for generous supply of angiotensin, and to AB Pharmacia, Uppsala, Sweden for generous supply of dextran.

References

Folkow, B., The efferent innervation of the cardiovascular system. *Verh. dtsch. Ges. KreislForsch.* 1959. *25.* 84—96.

Folkow, B., B. Johansson and S. Mellander, A comparison of the effects of noradrenaline and angiotensin on the resistance and the capacitance vessels in cats. *Acta physiol. scand.* 1960. *50.* Suppl. 175. 50—51.

Folkow, B., S. Mellander and B. Öberg. The range of effect of the sympathetic vasodilator fibres with regard to consecutive sections of the muscle vessels. *Acta physiol. scand.* 1961. *53.* 7—22.

Mellander, S., Comparative studies on the adrenergic neuro-hormonal control of resistance and capacitance blood vessels in the cat. *Acta physiol. scand.* 1960. *50.* Suppl. 176. 1—86.

Schachter, M., Polypeptides which affect smooth muscles and blood vessels, Pergamon Press Ltd. London 1960, p. 95—160.

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Autoregulation and Basal Tone in Consecutive Vascular Sections of the Skeletal Muscles in Reserpine-treated Cats

By

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Abstract

FOLKOW, B. and B. ÖBERG. *Autoregulation and basal tone in consecutive vascular sections of the skeletal muscles in reserpine-treated cats.* Acta physiol. scand. 1961. *53.* 105—113. — An experimental analysis has been performed in reserpine-treated cats concerning the extent of basal vascular tone and the nature of smooth muscle 'autoregulation' within the consecutive sections of the vascular bed of the skeletal muscles. The experiments suggest that these events are manifestations of a truly myogenic activity, basically independent of extrinsic sources of excitatory agents, and that it is essentially concentrated to the precapillary resistance vessels. Some implications of these data with regard to the cardiovascular system as a whole are briefly discussed.

Recent studies indicate that 'basal vascular tone', — *i. e.* the average extent of vasoconstriction, still present after elimination of the vasomotor nerves, — is a matter of local mechanisms and in principle independent of bloodborne vasoactive substances (see *e. g.* LÖFVING and MELLANDER 1956). The precise nature of these local mechanisms is, however, debated. A myogenic automaticity of the contractile elements, to some extent facilitated by the distending effect of the transmural pressure, appears to be the most reasonable explanation of the basal vascular tone. To this should be added the vascular sensitivity to any variation of smooth muscle environment, whether this is caused by a pressure-induced change in blood flow, by a shift of tissue metabolism or by a changed blood composition. — Vascular circuits, exhibiting a more pronounced

basal tone, which is modified by the mentioned factors, will also exhibit an often considerable 'autoregulation' of blood flow. By 'autoregulation' is implied the locally induced adaptations of vascular tone, which, for instance, tend to keep blood flow fairly constant independent of changes in perfusion pressure. — Some aspects of these problems are more fully discussed elsewere (FOLKOW and LÖFVING 1956, FOLKOW 1960).

It is, however, not yet disproved beyond doubt that locally released constrictor agents and regional reflex arcs might after all constitute the initiating mechanisms. Several authors still claim the existence of independent nerve plexa in the vascular walls, and reactions like the constrictor responses to venous distension have been ascribed to a local 'veni-vasomotor reflex' (GASKELL and BURTON 1953). Further, there is now good evidence of scattered chromaffin cells along the vessels (*e. g.* ADAMS-RAY et al. 1958), and such cells might act as local releasers of constrictor agents, such as catechol amines, thus establishing a tonic vascular contraction. — Such a concept implies, however, a fundamentally different mechanism as compared to the muscle automaticity view, as the initiation of basal vascular tone should then not originate within the contractile elements themselves, but be a matter of extrinsic though local stimuli. In case this latter view should be correct, basal tone and autoregulation could be expected to be largely eliminated in reserpine-treated animals, as it is known that this drug is able to deplete the organism of catechol amines and related smooth muscle stimulating substances.

Experiments have therefore been performed on reserpine-treated cats in order to evaluate the extent of basal vascular tone and blood flow autoregulation in skeletal muscles. A technique has been utilized, which allows a simultaneous recording of the responses of the pre- and postcapillary resistance vessels and the capacitance vessels, making it possible to find out whether basal tone and autoregulation of the vascular smooth muscles is of similar degree in all parts of a vascular circuit or essentially confined to some of its consecutive sections. Such a possibility is of some principal interest as it provides information concerning the extent of functional differentiation inbetween different parts of a vascular circuit with respect to their contractile elements.

Method

Medium-sized cats were used, which during four days prior to the experiment had been treated with reserpine (Serpasil®) given intramuscularly in daily doses of 2 mg per kg. The animals were anesthetized with a combination of chloralose, 50—60 mg per kg, and urethane, 100—150 mg per kg. — The technique, utilized for a separate recording of the reactions of the pre- and postcapillary resistance vessels and the capacitance vessels, has previously been described and analysed in detail (MELLANDER 1960) and is outlined in principle in another paper in the present volume (FOLKOW, MELLANDER and ÖBERG 1961). For the present purpose it should only be added that the venous outflow pressure from the hind parts could be set to any wished level, and further, also the filling pressure of the heart could be easily adjusted. In these animals, entirely deprived

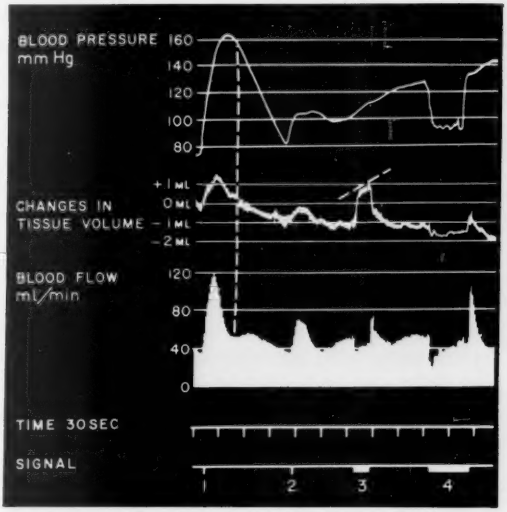

Fig. 1. Reserpine-treated cat, 3.4 kg. Chloralose-urethane. The figure illustrates the effects on blood flow and 'blood volume' when arterial blood pressure is temporarily raised 90 mm Hg (1), 25 mm Hg (2), when venous pressure is raised 10 mm Hg (3) and when arterial blood pressure is lowered 40 mm Hg (4).

of functioning vasoconstrictor and accelerans nerves and with acutely cut vagal nerves, all cardiovascular reflex adjustments were eliminated. Therefore, even minor shifts of venous return from the funnel to the heart markedly affected its stroke volume, and hence cardiac output, making it easy to induce even drastic shifts in arterial blood pressure simply by changing the inflow rate from the funnel. Alternatively, careful adjustments of this inflow made it possible to keep the arterial pressure fairly canstant, if so wanted.

In order to exclude the skin blood flow in the hind limbs as much as possible, tight ligatures were placed at the proximal ends of the paws and the tail. Generally 80—85 per cent of the studied tissue volume was found to be made up by skeletal muscles. In a few cases both hind limbs were also cautiously skinned. To maintain a steady state with regard to ventilation, constant artifical respiration was given to such an extent as to barely suppress the spontaneous ventilation of the anesthetized animal.

With the method used the hind parts constituted a muscle vascular bed, entirely deprive of all adrenergic nervous connections, but normally perfused from the upper half of the animal, which merely acted as a physiological blood pump and oxygenator. Both the arterial inflow pressure and the venous outflow pressure could be adjusted to any wished level, allowing an analysis of the influence of arterial and venous pressure changes on both flow and regional blood volume and, in certain circumstances, also on transcapillary filtration exchange.

Results

The vascular reactions to different types of pressure changes, which with great regularity could be repeated, are best presented by describing in detail a characteristic experiment (Fig. 1). At '1' and on smaller scale at '2' in the figure, a steep and marked increase of arterial pressure is induced and its effect on blood flow and blood volume can be followed. It is clear from the figure that both

flow, and also volume, increases in rough proportion to the pressure rise, to start with. However, while the arterial pressure rise is still at its top level both blood flow and volume increases fade away rapidly and return almost to their initial values. Obviously a pronounced vasoconstriction is initiated after a brief latency as a response to the pressure rise and, when fully established this vascular 'autoregulation' is almost able to normalize both flow and volume, in spite of the fact that the arterial pressure is about doubled in '1'. — Such a pronounced arterial pressure rise would, if vascular tone had remained the same, have raised also mean capillary and venous pressures considerably, causing a lasting, pronounced tissue volume increase, due to both an outward filtration and venous distension. No such phenomenon takes place, however. On the contrary, the initial volume increase vanishes as rapidly as does the flow increase. This fact suggests that the marked reactive increase of flow resistance takes place mainly within the precapillary resistance section, which has the hemodynamic consequence that the arterial pressure rise is no longer transmitted to the capillary and venous sections. It is, however, so far still possible that some increase of postcapillary flow resistance takes place, but '3' in Fig. 1 makes it clear that a pressure increase within the postcapillary, venous section of the vascular bed will not cause any significant increase of smooth muscle tone in this section. — Arterial blood pressure is here kept fairly constant, but venous outflow pressure is instead suddenly increased some 10 mm Hg, simply by raising the level of the Gaddum recorder 13—14 cm. The vascular response to this manoeuvre is an immediate and rather marked volume increase, obviously due to venous distension, and this pooling of blood reduces, of course, also the venous outflow for a few seconds. However, this rapid phase of venous distension is soon completed, and is then followed by a much slower, though very constant volume increase. It is clear from earlier analyses of such phenomena (see MELLANDER 1960) that this second slope of volume increase is a matter of outward capillary filtration, and this process keeps on at a surprisingly steady rate also when the period of raised venous pressure is very long. It should here especially be observed that raising the venous pressure does not induce any significant reactive tone changes within the venous section; the passive distension remains unhampered and so does the rate of outward filtration, which should not have been the case if reactive shifts in venous tone had occurred. — From '2' and onwards in Fig. 1 the arterial pressure is again allowed to rise, but now only gradually. It is seen from the figure that both blood flow and volume are then capable to adapt themselves continuously, so as to counteract the influence of the rising arterial pressure head without any noticeable time lag. — In '4' the opposite of '1' is induced: Arterial pressure is abruptly decreased by a partial occlusion of the abdominal aorta and kept at the lowered level for a short period of time. It is seen from the flow and volume tracings that the immediate effect of the arterial pressure drop is a decrease of both flow and volume. However, after a latency of only some 5—10 seconds they again increase slowly,

in spite of a constant arterial and venous pressure, indicating a progressive vascular relaxation, presumably also here affecting mainly the precapillary resistance vessels. This relaxation is drastically unmasked when arterial pressure is suddenly restituted, leading to a marked, but brief reactive hyperaemia and also to a volume increase.

The potency of these 'autoregulation' phenomena in reserpine-treated cats are, as seen, remarkable both concerning extent and rate; the extent of autoregulation was in fact often so striking that it, if anything, was superior to that seen in sensitively reacting vascular beds of the acutely denervated cat's limb. These experiments also made it clear, that basal vascular tone is essentially of normal range in reserpine-treated animals, which is obvious from the flow figures of 6—8 ml/min/100 ml of tissue for resting, denervated skeletal muscles, which were recorded as long as arterial pressure was kept within its normal range. It was also clear from the fact that intraarterial injections of big doses of acetylcholine increased blood flow some five times. As a contrast to the precapillary resistance vessels, the venous side of the circulation, constituting the postcapillary resistance vessels and the main part of the capacitance vessels, had, —beside its evidently poor or absent autoregulation, — a very low basal tone. This was obvious from the fact that supramaximal amounts of intraarterially injected acetylcholine, which induced often fivefold increases of blood flow, calculated to correspond to about 40 % increase of internal radius of the resistance vessels, only increased the regional blood volume roughly 15—20 per cent, as calculated from MELLANDER's measurements of the blood volume in the hind part (1960). This corresponds to about 10 % increase of internal radius of the capacitance vessels. Further, much of the blood volume increase must have been due to a passive distension of the veins and to the unavoidable increase of blood content within the dilated precapillary resistance vessels. The lack of further volume increases could not be due to the fact that the dilator drug did not reach the venous side or lacked the power to relax the veins, because if venous tone had been artificially raised on beforehand by noradrenaline infusion, acetylcholine was able to induce very drastic increases of regional blood volume. — The fact that the present animals really were more or less completely depleted of catechol amines was proved by stimulations of the abdominal sympathetic trunks after that the symphatetic dilator fibres had been blocked by atropine. Even maximal excitations at highest physiological rates, which normally increases flow resistance some 7—8 times and decreases the regional blood volume about 30 per cent, — about 6—8 ml in a normal size cat (MELLANDER 1960), — had only a very minute or no effect on flow and volume.

Thus the experimental data, so far described, make it clear that catechol-depleted animals exhibit a pronounced basal tone and autoregulation, but that these manifestations of inherent smooth muscle activity were essentially restricted to the precapillary resistance vessels. Whether the autoregulation of the precapillary resistance vessels is only a matter of alternations in smooth muscle

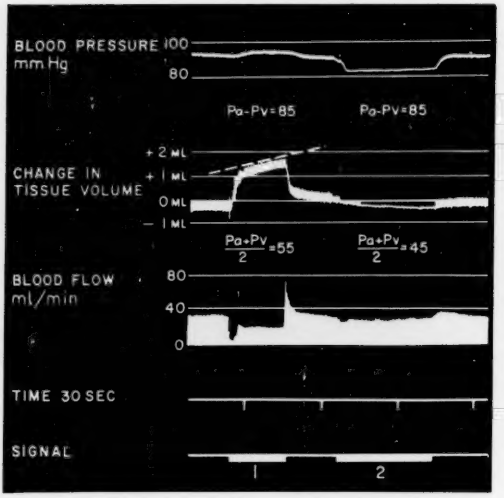

Fig. 2. Reserpine-treated cat, 3.0 kg. Chloralose-urethane. 'P_a' in the figure denotes arterial inflow pressure, 'P_v' venous outflow pressure of the isolated hind part. — The figure illustrates the effects on blood flow and 'blood volume' when mean intravascular pressure $\frac{(Pa + Pv)}{2}$ is raised (1) and lowered (2) while the perfusion pressure (Pa—Pv) is kept the same.

environment, due to the flow changes enforced upon the tissue by the primary shifts in perfusion pressure, or whether also changes in transmural pressure as such contributes, is illustrated in Fig. 2. In '1' and '2' of this figure the pressure head is slightly lowered to reach very nearly the same level, 85 mm Hg, in the two cases. However, in '1' the pressure head is reduced by raising the venous pressure about 10 mm Hg, which implies a *raised* mean transmural pressure; in '2' the pressure head is reduced by lowering the arterial pressure, and hence also mean transmural pressure is decreased. It is easily calculated that the mean intravascular pressure, $\frac{(Pa + Pv)}{2}$, is about 55 and 45 mm Hg, respectively, in the two cases. — If it is assumed as a reasonable figure that roughly 80 per cent of the flow resistance is made up by the precapillary vascular section and that tissue pressure is low and uniform, it can be deduced that the average transmural distending pressure also within the precapillary resistance vessels is some 20 per cent higher in '1' than in '2'. In spite of the fact that the pressure head is the same and the chances of passive-elastic vascular distension are greater in '1', flow resistance is, in fact, almost 30 per cent *higher* in alternative '1', where the transmural pressure is raised. Local 'veni-vasomotor reflexes' cannot possibly have been operating in these animals, depleted of the constrictor fibre transmitter. Further, had the chemical environment of the smooth muscles been alone responsible of autoregulation, the blood flow would, if anything, have been slightly bigger in '1' than in '2'. Finally, having the animal

to breath a gas-mixture containing 6 % oxygen, implying a lowering of arterial oxygen saturation to about 50—55 %, this rather drastic change in blood composition and hence in vascular smooth muscle environment, was as a rule followed by comparatively small shifts in vascular tone in the present circumstances, and these shifts were not observed until after a considerable latency. These circumstances suggest that changes in the chemical environment of the vascular smooth muscles do not form the only, possibly not even the major mechanism responsible of autoregulation. The distension of the contractile elements in the vascular walls, offered by the transmural pressure, appears to be of considerable importance in such vessels which exhibit a more pronounced basal tone.

Discussion

The present experiments make it highly unlikely that any lokal nerve plexa or cell structures, producing vasoconstrictor agents, should be the background of basal vascular tone and autoregulation of flow in skeletal muscles. In fact, the reserpine treatment appears to have, if anything, potentiated these inherent reactions of the vascular contractile elements, presumably because reserpine treatment implies a 'pharmacological denervation', which is known to give rise to a sensitization of the effector cells to a variety of stimuli (EMMELIN and MUREN 1951). The experiments thus strengthen the view that both basal vascular tone and autoregulation of flow are manifestations of an inherent, myogenic activity. It appears likely that this is the case also in other vascular regions exhibiting autoregulation of flow, *e. g.* the kidneys.

It can also be concluded from the present experiments that the distension, offered by the blood pressure, acts as a facilitating influence on the myogenic activity of the vessels, presumably by increasing the *rate* of spontaneous discharge of the individual muscle cell as has been observed for myogenically active intestinal smooth muscles (BÜLBRING 1955). If the frequency of the spontaneous contractions is raised, the time fraction occupied by the relaxation phase can be expexted to be proportionally shortened, and the integrated result of this would be a somewhat raised vascular tone. — Obviously the influence of distension is only significant in vascular sections, which really exhibit a pronounced basal tone, *i. e.* in the precapillary resistance vessels. Where myogenic automaticity is negligible or absent, there is nothing that could be facilitated by distension.

It is quite obvious, however, that also shifts in the chemical environment of the vascular smooth muscle cells profoundly affect their tone. In some situations the influence of such changes will cooperate with the effects of shifts in transmural pressure, as in the establishment of autoregulation of flow. In other circumstances the two mentioned factors will counteract each others, for instance when blood flow is decreased by way of a raised venous pressure, or where blood pressure and tissue activity are concomitantly increased. What

the net effect on vascular tone will be in such situations may vary from one tissue to the other. It can be expected to depend on the relative sensitivity of the regional smooth muscles, — which is not necessarily the same in all tissues, — to the contrary-directed shifts in transmural pressure and chemical environment. Thus, even if only these two mechanisms out of the manifold that affect vascular tone are considered, one deals in fact with a highly complex integration, where the balance may vary, not only inbetween the different vascular circuits, but even within one and same circuit from time to time. See also the recent study by STAINSBY and RENKIN (1961), where autoregulation of muscle blood flow is discussed from a viewpoint based on a somewhat different experimental approach. — Too little is still known about the fundamental characteristics of the contractile elements of the small blood vessels to justify a more detailed discussion of these problems at present. It should be emphasized, however, that in looking at the control of blood flow in relation to tissue metabolism, nervous control etc. in the intact organism, the combined action of distension and metabolic influences on the vascular smooth muscles should constantly be kept in mind; too often only nervous and hormonal excitatory factors are considered.

It is of interest that the pronounced basal tone and autoregulation are essentially manifestations of only the precapillary resistance vessels from the point of view that this implies a clearcut smooth muscle differentiation inbetween the various consecutive sections of the vascular bed. Among other things such a mechanism tends to maintain mean capillary pressure, and hence filtration exchange, more constant in vascular regions exposed to *e. g.* changes in hydrostatic pressure. When shifting from a recumbent to an erect position the transmural capillary pressure in the legs would be much increased with a consequent rapid edema formation, if no compensatory reactions take place. A strictly locally controlled adjustment, like the mentioned myogenic autoregulation of tone in the precapillary resistance vessels, will here add to other compensation mechanisms that tend to counteract the shifts in filtration exchange. It will also automatically compensate for such regional flow increases that would have occurred by way of the distension of the precapillary resistance vessels when the transmural pressure is increased. Lastly, the present findings may explain why sympathetomized subjects, being not too much disturbed in their regulation of regional and total flow resistance, practically always are much troubled by a poor control of the veins. This is obvious by the prompt pooling of blood on the venous side by even slight increases of hydrostatic pressure in such subjects, which consequently affects the venous return to the heart. The present experiments suggest that both the basal tone and the power of autoregulatory adjustments are insignificant on the venous side, when taken as a unit. Obviously this most important section of the cardiovascular system is in a poor state what concerns its compensatory adjustments, once its extrinsic neurogenic control is eliminated.

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References

Adams-Ray, J., H. Nordenstam and J. Rhodin, Über die chromaffinen Zellen der menschlichen Haut. *Acta Neuroveg.* (Wien) 1958. *18.* 304—309.

Bülbring, E., Correlation between membrane potential, spike discharge and tension in smooth muscle. J. *Physiol.* (Lond.) 1955. *128.* 200—221.

Emmelin, N. and A. Muren, Paralytic secretion in cats after treatment with atropine. *Acta physiol. scand.* 1951. *22.* 277—280.

Folkow, B., Role of the nervous system in the control of vascular tone. Circulation (N. Y.) 1960. *21.* 760—768.

Folkow, B. and B. Löfving, The distensibility of the systemic resistance vessels. *Acta physiol. scand.* 1956. *38.* 37—52.

Folkow, B., S. Mellander and B. Öberg, The range of effect of the symphathetic vasodilator fibres with regard to consecutive sections of the muscle vessels. *Acta physiol. scand.* 1961. *53.* 7—22.

Gaskell, P. and A. C. Burton, Local postural vasomotor reflexes arising from the limb veins. *Circ. Res.* 1953. *1.* 27—39.

Löfving, B. and S. Mellander, Some aspects of the basal tone of the blood vessels. *Acta physiol. scand.* 1956. *37.* 134—141.

Mellander, S., Comparative studies on the adrenergic neurohormonal control of resistance and capacitance blood vessels in the cat. *Acta physiol. scand.* 1960. *50.* Suppl. 176. 1—86.

Stainsby, W. N. and E. M. Renkin, Autoregulation of blood flow in resting skeletal muscle. Personal communication. To be published.

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Measurement of the Oxygen Diffusion Coefficient in the Subcutis of Man

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Abstract

CALIGARA, F. and G. ROOTH. *Measurement of the oxygen diffusion coefficient in the subcutis of man.* Acta physiol. scand. 1961. *53.* 114—127. — The subcutaneous tissue is considered a ball which is surrounded by a network of capillaries. By applying general equations for the diffusion of gases a series of equations are evolved. These equations show the importance of the different factors which influence the oxygen supply to the subcutaneous tissue. Some preliminary measurements are done in order to show the validity of these equations, and in order to show how they may be used most conveniently. The increase in tissue oxygen tension when a subject is switched over from breathing air to breathing pure oxygen is measured with a covered Pt electrode. From the resulting curve the oxygen diffusion coefficient is calculated. If the oxygen consumption of the tissue and the intercapillary distance is known the capillary oxygen tension and the blood flow may also be calculated. The results obtained are of the expected order of magnitude.

MONTGOMERY and HORWITZ (1950) studied how the subcutaneous oxygen tension changes during inhalation of oxygen. They measured the oxygen tension (p_{O_2}) with a platinum electrode connected to a suitable circuit and recording device. This technique has been used under various experimental and clinical conditions, see MONTGOMERY (1957).

It could also, for instance, be a suitable technique for studying the increase of the tissue oxygen tension p_T when a newborn infant begins to breath but before

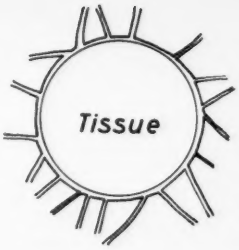

Fig. 1. The model of the oxygen supply to the tissue.

starting any series of measurements we wanted to try to evaluate the various factors that might affect the measurements. In a repetition of Montgomery's experiments, a subject was given pure oxygen to breathe. The subcutaneous tissue oxygen pressure increased to a maximum and decreased again to the initial level when the oxygen was withheld. It was observed that the two branches of the curves could not be superimposed. Now it is known that an increase in arterial oxygen tension p_a produces contraction in the vessels of the eyes, CUSICK *et al.* (1940), brain, TINEL (1927), COBB and FREEMONT-SMITH (1931) and kidneys, FISHMAN *et al.* (1951) and decrease in oxygen tension produces a dilatation. The skin, like the fundus oculi becomes pale, when a new born infant is given pure oxygen. This vasomotor reaction reduces the speed of the increase in tissue oxygen tension, and it also increases the speed with which the same tension is reduced when oxygen is withdrawn. These were both phenomena which could be observed in our curves. An interpretation of the curves of the tissue oxygen tension should therefore give a mathematical expression of the oxygen supply to the tissues and of the various factors involved. In order to achieve this, preliminary studies indicated that the arterial oxygen tension (p_a) must also be known. To show the uses of the equations evolved, some preliminary experiments have been performed and their results have been used mainly to demonstrate how to apply the equations to practical data. Thus the oxygen diffusion coefficient, which has so far only been determined in excised pieces of tissue, is experimentally determined in vivo. Also, indicative figures are obtained for the mean capillary oxygen tension, the blood flow per volume of tissue and oxygen vasomotor reaction. Although the two latter entities may be obtained by quite different measurements, it may be of value to draw as much information as possible from the arterial and tissue oxygen tensions changes.

I. Mathematical Treatment

From the tissue oxygen pressure (p_T) curve, only the oxygen diffusion coefficient can be determined. But, as will be shown, it is possible, with the use of established formulas for diffusion, to determine the factors governing the tissue p_{O_2}, *i. e.*, the p_{O_2} of the capillaries and the blood flow if the p_T and p_a are determined at the same time.

TABLE 1.

Symbols

p_T	Oxygen tissue pressure.
p_{Ti} or $p_{T(t=o)}$	Initial oxygen tissue pressure.
p_c	Capillary oxygen pressure.
p_e	Mean capillary oxygen pressure.
a	Radius of the sphere.
r	Distance from the center of the sphere to the point of measurement.
D	Oxygen diffusion coefficient.
t	Time
m	Reaction rate of the process consuming oxygen.
α	Solubility of oxygen in the tissue.
γ	Solubility of oxygen in plasma.
γ'	Solubility of oxygen in whole blood.
Δp_e	Pressure drop needed for the metabolic requirement of the tissue.
Δp_{ef}	Final increase in capillary and tissue pressure.
p_a	Arterial oxygen pressure.
P_1	Final increase in the arterial oxygen pressure.
P_2	Initial arterial oxygen pressure.
P_3	Conventional zero level for capillary oxygen pressure.
$p_{(a-\bar{e})}$	Difference between arterial and capillary oxygen pressure.
Q_1	Amount of oxygen consumed by the tissue metabolism.
Q_2	Amount of oxygen required to increase the tissue oxygen pressure.
F	Blood flow.
$OVMR$	Oxygen vasomotor reaction.

The Model

Because of the abundance of capillaries in the subcutaneous tissue we have assumed a model consisting of a net-work of capillaries forming a sphere and containing tissue within it. The capillaries are equivalent to the white coating of a golf-ball as it were, and the tissue corresponds to the substance inside. Thus the oxygen diffuses from the surface of a sphere to the interior. The model is represented in Fig. 1. The oxygen tension in the capillaries (p_c) is of course different at various distances from the artery, but we will only consider the mean capillary pressure ($p_{\bar{e}}$) as being uniform around the sphere.

The traditional model used for studying the diffusion of oxygen from the vessels into the tissues is Krogh's cylinder (1919). For the following reasons we have decided to use the golf-ball model:

1. The anatomical distribution of the capillaries in the subcutaneous tissue agrees better with our model than with the Krogh-cylinder.

2. The Krogh-model considers only one capillary and its surrounding cylinder and disregards the influences of crossing vessels.

3. Even if the Krogh-model is extended to take several cylinders into account (THEWS 1953), it is assumed that they are of an equal distance from arteries.

4. The Krogh-model assumes that the capillaries are rectilinear.

The mathematical treatment will of course be different if a measurement of tissue p_{O_2} is made at one geometrical point of the sphere or if the measurement itself gives a mean value over a surface which is large in relation to the size of the sphere. We will begin by treating the electrode as an ideal point.

The ideal pointlike electrode:

The symbols used are summarized in Table 1.

If, for the moment, the oxygen consumption of the tissue is disregarded, we may use Crank's formula (1956) which gives the pressure at a distance r from the center of the sphere at the time t,

$$\frac{p_{T(r,t)} - p_{Ti}}{p_e - p_{Ti}} = 1 + \frac{2a}{\pi r} \sum_{n=1}^{\infty} \frac{(-1)^n}{n} \sin\left(\frac{n\pi r}{a}\right) e^{-Dn^2\pi^2 t/a^2} \tag{1}$$

where:

$p_{T(r,t)}$ = p_{O_2} in the tissue at the point r at the time t.
p_{Ti} = the uniform initial p_{O_2} in the tissue independent of t and r.
(For other symbols see Table I).

When a steady-state is reached, that is at $t = \infty$, the exponential part of (1) equals zero and the equation is reduced to

$$\frac{p_{T(r)} - p_{Ti}}{p_e - p_{Ti}} = 1 \tag{1 a}$$

This equation is only fulfilled if $p_{T(r)} = p_e$.

It follows then from a study of this equation that, if there is no oxygen consumption, in a steady-state, the p_{O_2} inside the sphere is the same everywhere and equals the pressure in the capillaries.

As the tissue through which the oxygen diffuses, consumes oxygen, it is necessary to introduce a factor for the metabolic oxygen consumption ROUGHTON (1952) studied the problem of the diffusion of a gas into a sphere where at the same time the gas is undergoing a zero order reaction by which it is consumed. Using the same symbols as before his equation is:

$$p_{T(r,t)} = p_e - \frac{m(a^2 - r^2)}{6D} + \\ + \frac{2a}{\pi r} \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n} \left[\frac{ma^2}{Dn^2\pi^2} - (p_e - p_{Ti})\right] \sin\left(\frac{n\pi r}{a}\right) e^{-Dn^2\pi^2 t/\alpha a^2} \tag{2}$$

where:

m = the reaction rate of the process consuming oxygen.
α = the solubility of oxygen in the tissue.

This equation is valid if the amount of oxygen consumed by the tissue is independent of the p_{O_2} (zero order reaction). CHANCE (1957) has shown that this is the case over a wide range of pressure, and it is assumed in the present work that m is independent of p_{O_2}.

At a steady state ($t = \infty$) the exponential part of (2) equals zero and the equation is reduced to:

$$p_{T(r)} = p_e - \frac{m(a^2 - r^2)}{6D} \tag{2 a}$$

Equation (2 a) is symmetrical with respect to the center of the sphere which means that the p_{O_2} is constant over any concentric spherical surface within the sphere. It may also

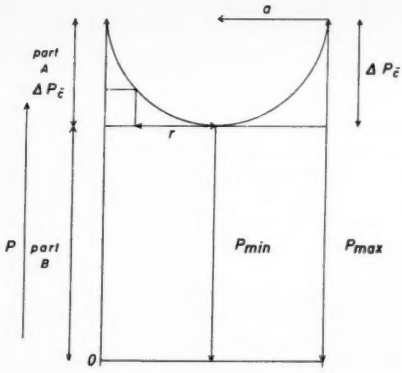

Fig. 2. The meniscus represents the p_{O_2} along a diameter. This p_{O_2} goes from a mininum p_{min} at the center to a maximum p_{max} at the periphery. The p_T which is measured may assume any value within these limits depending upon where inside the sphere the point of measurement happens to be placed.

be seen from (2 a) that when r decreases p_T also decreases and that p_T has a minimum at the center of the sphere, where r is zero, and a maximum at the surface where $r = a$.

From (2 a) it is evident that at the periphery of the sphere, where $r = a$, also $p_T = p_{\bar{e}}$ *i. e.*, the tissue pressure is the same as the mean capillary pressure. The difference between the highest p_T (p_{max}) at the periphery of the sphere and the lowest p_T (p_{min}) in the centre of the sphere ($p_{max} - p_{min}$) we call $\Delta\, p_{\bar{e}}$. The shape of the meniscus in fig. 2 and the value of $\Delta\, p_{\bar{e}}$ are given by the second term of the right-hand member of the equation (2 a) and are therefore not affected by the absolute value of the p_{O_2}. $\Delta\, p_{\bar{e}}$ represents the pressure required to furnish all and only the amount of oxygen necessary to the tissue for its own metabolic requirements and it depends upon the reaction rate (m), the diffusion coefficient (D) and a geometrical factor, which in our case is the radius a.

It follows that changes in the capillary pressure will neither affect the value of $\Delta\, p_{\bar{e}}$ nor the shape of the meniscus, unless the mean capillary pressure itself becomes lower than $\Delta\, p_{\bar{e}}$. For physiological reasons it may be assumed that $p_{\bar{e}}$ never becomes lower than $\Delta\, p_{\bar{e}}$.

In order to calculate the various factors governing the transportation of oxygen to the tissue it is convenient to divide the tissue pressure and the capillary pressure into two reciprocally corresponding parts, part *A* and part *B*.

Part A in the capillaries is $\Delta\, p_{\bar{e}}$ and in the tissue may assume any value between zero and $\Delta\, p_{\bar{e}}$ depending upon the distance from the center of the sphere. As has been stated $\Delta\, p_{\bar{e}}$ represents the pressure needed to furnish the tissues with their metabolic requirements. Once the tissue obtains the oxygen it needs for its metabolism, part A is in a steady state and equation (2) therefore is simplified to equation (2 a). On further examination of equation (2 a) and Fig. 2, it will be remembered that $p_{\bar{e}} = p_{max} = p_{T_{(r)r=a}}$ and $p_{min} = p_{T_{(r)r=0}}$. Introducing $r = 0$ and $r = a$ into (2 a) and subtracting we get:

$$\Delta\, p_{\bar{e}} = p_{max} - p_{min} = \frac{m a^2}{6 D} \tag{3}$$

Part B is that part which is not influenced by the oxygen consumption of the tissue and is governed by equation (1) or (1 a). This part is independent of r and t in a steady state but dependent on them in a dynamic state. The corresponding part of the capillary pressure is $(p_{\bar{e}} - \Delta\, p_{\bar{e}})$ as $\Delta\, p_{\bar{e}}$ was part *A* governed by the oxygen consumption. Any

change in the capillary pressure is reflected only in this part B of the tissue pressure. From (1 a) it will be seen that, in a steady state, part B of the tissue pressure is the same as the corresponding capillary part and is therefore equal to $(p_c - \Delta p_c)$.

The information available in a steady state is not adequate to solve the equations, whereas in a dynamic condition a sufficient amount of data may be obtained. Such a condition would occur if the p_a increased, as during the breathing of oxygen. Thus, in order to calculate the capillary pressure we must find a mathematical relationship between the tissue pressure and the capillary pressure in dynamic conditions.

The increase in the arterial oxygen pressure when pure oxygen is breathed must be governed by a simple exponential function of time as it depends upon the washing out of nitrogen from the alveoli.

$$p_a(t) = P_1 (1 - e^{-\delta t}) + P_2 \tag{4}$$

where:

P_1 = difference between the initial and the final pressure.
P_2 = initial pressure.
δ = a constant.

This equation we have found experimentally.

The constants P_1, P_2 and δ may be determined from measurement of the p_a during oxygen breathing. When the pressure in the arterial blood has an exponential form, it seems reasonable to expect a similar form for the oxygen pressure in the capillaries. It is assumed that the oxygen tension in the capillaries is governed by an equation of the same type as (4) but with different constants.

The equation is:

$$p_c(t) = \Delta p_{cf} (1 - e^{-\beta t}) + P_3 \tag{5}$$

where:

Δp_{cf} = the difference between the initial and the final pressure which, as is shown in Fig. 3, is equal to the total increase in the tissue pressure and may therefore be determined experimentally.
β = a constant.
P_3 = another constant representing the initial pressure.

If the p_{O_2} in the capillaries is governed by equation (5), then p_{O_2} inside the tissue sphere, is governed by another equation, CRANK (1956).

$$p_T(r,t) = \Delta p_{cf} - \frac{\Delta P_{cf} \cdot a}{r} e^{-\beta t} \frac{\sin \sqrt{\frac{\beta a^2}{D}} \cdot \frac{r}{a}}{\sin \sqrt{\frac{\beta a^2}{D}}} - \tag{6}$$

$$- \frac{\Delta p_{cf} \cdot 2 \beta a^3}{\pi D r} \sum_{n=1}^{\infty} (-1)^n \frac{e^{-Dn^2\pi^2 t/a^2}}{n(n^2\pi^2 - \beta a^2/D)} \sin\left(\frac{n\pi r}{a}\right)$$

(6) does not give the absolute pressure, but only the increase from the initial steady state. The initial pressure corresponding to P_3 in (5), must be added to (6) in order to represent the total tissue pressure. p_T in (6), therefore, has a slightly different meaning than in the other equations, but it might be confusing to introduce a new symbol for the tissue pressure in this equation.

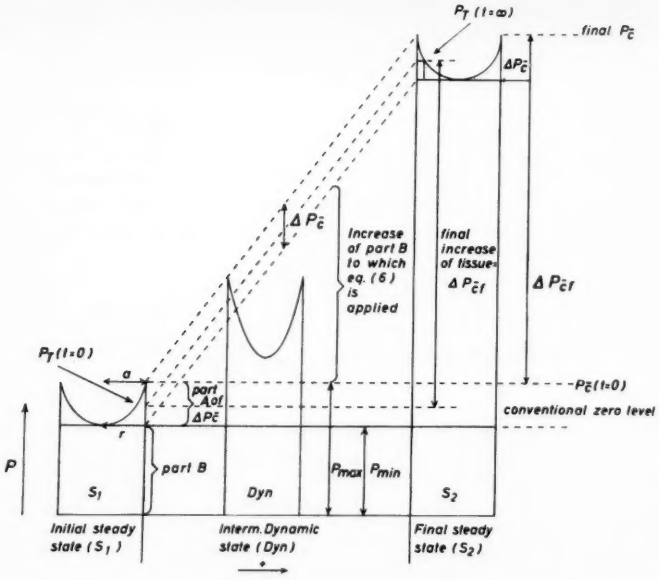

Fig. 3 is a schematic representation of the tissue pressure changes during oxygen inhalation.

There is to the left an initial steady state (S_1) and to the right a final steady state (S_2), in between which there is a continuous series of dynamic states, one of which is represented as Dyn.

Because of the steady state in S_1 and S_2 the shape of the menisci are equal as shown in the Fig. It then follows from geometrical considerations that $\Delta\, p_{\bar{e}f}$ = total increase in the tissue pressure. In the intermediate dynamic state the shape of the meniscus is altered because it is formed by the original shape of the meniscus and a superimposed meniscus due to the flow into the sphere of oxygen needed for an increase in tissue pressure.

Equation (6) is applied to the part indicated.

In order to get $p_{\bar{e}}$, part A and $p_{\min}$ must be added to the pressure from equation (6). $p_{\min}$ is the initial Part B. Or in other words: The final mean capillary pressure is obtained by adding the observed increase in tissue pressure to the initial tissue pressure which consists of part A + part B.

P_3 in (5) is used as a conventional zero level and is the $p_{\bar{e}}$ of the initial steady state.

Equation (6) shows that p_T (r,t) is made up from a summation of exponential terms. Before we can apply (6), it should be shown that an experimental p_T curve fulfills such conditions, and in attempts to rectify the p_T curve we found this to be the case.

In order to calculate $p_{\bar{e}}$, β must be known and this is accomplished by solving (6). If for the sake of convenience we write (6) as $h\,(t)$ then for $t = t_1$ we get:

$$p_{T(t=t_1)} - p_{T(t=0)} = h_{(t_1)} \qquad (7)$$

(7) contains 3 unknowns, namely r, D, and β. If we establish a set of 3 equations (7) for different values of t the system may be solved and the constants determined.

In order to solve (5) and obtain $p_{\bar{e}}$ only P_3 remains.

As mentioned P_3 is $p_{\bar{e}(t=o)}$. From (2 a) it follows that

$$p_{\bar{e}(t=o)} = p_{T(t=o)} + \frac{m(a^2 - r^2)}{6D} = P_3 \tag{8}$$

This can only be used if m and a are known from independent measurements whereas r and D, as just stated, are obtained from (7). P_3 is then calculated and from this $p_{\bar{e}}$ in (5).

The blood flow may be calculated from the amount of oxygen which enters the tissue. In order to obtain this several steps are needed.

The difference between the brachial artery p_{O_2} and the p_{O_2} in the capillaries ($p_{(a-\bar{e})}$) is then given by (4) subtracted from (5) that is by

$$p_{(a-\bar{e})(t)} = P_1(1 - e^{-\delta t}) + P_2 - \Delta p_{\bar{e}f}(1 - e^{-\beta t}) - P_3 \tag{9}$$

The amount of oxygen that enters the surface of the tissue sphere per unit of time is given by two terms equivalent to parts A and B: one corresponding to the oxygen consumption of the tissue, which is constant and given by

$$\frac{\partial Q_1}{\partial t} = 4\pi m a^3/_3 \tag{10}$$

and the other corresponding to the oxygen needed for increasing the pressure inside the sphere. This is calculated by multiplying (6) by α, the solubility of oxygen in the tissue, which gives the oxygen concentration in function of t and r. If we multiply this by $4\pi r^2$ and integrate with respect to r within the limits o and a, we obtain the amount of oxygen present in the sphere in function of time. Then by derivating with respect to t, we obtain the amount of oxygen entering the surface of the sphere per unit of time in function of time. The result of all these operations is:

$$\frac{\partial Q_2}{\partial t} = 4\alpha\pi a \Delta p_{\bar{e}f} D\bar{e}^{-\beta t}\left[1 - \sqrt{\frac{\beta a^2}{D}} \cdot \cot\sqrt{\frac{\beta a^2}{D}}\right] - \\ - 8\alpha a^3 \Delta p_{\bar{e}f}\beta \sum_{n=1}^{\infty} \frac{\pi}{(n^2\pi^2 - \beta a^2/D)} e^{-Dn^2\pi^2 t/a^2} \tag{11}$$

Calling $Q(t)$ the total amount of oxygen entering the sphere per unit of time

$$\frac{\partial Q}{\partial t} = \frac{\partial Q_1}{\partial t} + \frac{\partial Q_2}{\partial t} \tag{12}$$

Calling $F(t)$ the blood flow through the capillaries per unit of time and γ the solubility of oxygen in the blood we have:

$$F_{(t)}\gamma\, p_{(a-\bar{e})(t)} = \frac{\partial Q}{\partial t} \tag{13}$$

and

$$F_{(t)} = \frac{\partial Q}{\partial t}/\gamma p_{(a-\bar{e})(t)} \tag{14}$$

When using equations (13) and (14) it must be remembered that γ may be considered constant only in limited parts of the dissociation curve.

Calling F_{air} the blood flow when breathing air, we may now define the oxygen vaso motor reaction *OVMR* as

$$OVMR = \frac{F_{air}}{F_{(t)}} \tag{15}$$

The actual electrode

The pointlike electrode only represents an ideal case which cannot at present be realized because the electrodes available have a size which has the same order of magnitude as the tissue sphere assumed as a model. Therefore, the treatment described above has to be modified in order to fit the experimental circumstances. If the active surface of the electrode is of the same order of magnitude as the maximum section of the sphere the pressure measured is not the pressure at one point of the sphere but an average pressure over all the sphere.

The tissue pressure read off with the electrode is then not the pressure given by (7) but is the pressure given by

$$\bar{p}_{T(t)} = \frac{1}{a} \int_o^a (7)\, dr \tag{7 a}$$

Since the direct integration expressed in (7 a) is not obtainable in finite terms the following procedure is adopted: Multiplying (7) by α (oxygen solubility in the tissue) we obtain the amount of oxygen present at r and multiplying again by $4\pi r^2$ we obtain the amount of oxygen present on the surface of a sphere with the radius r; now integrating with respect to r within the limits o and a we obtain the amount of oxygen present in the sphere; this divided by $\frac{4}{3}\pi a^3$ (volume of the sphere) and again by α gives the average pressure within the sphere, the result is:

$$\bar{p}_{T(t)} = \Delta P_{ef} - \frac{3\Delta p_{ef} D e^{-\beta t}}{\beta a^2}\left[1 - \left(\frac{\beta a^2}{D}\right)^{\frac{1}{2}} \cot\left(\frac{\beta a^2}{D}\right)^{\frac{1}{2}}\right] + \\ + \frac{6\Delta p_{ef}\beta a^2}{\pi^2 D} \sum_{n=1}^{\infty} \frac{e^{-Dn^2\pi^2 t/a^2}}{n^2\left(n^2\pi^2 - \frac{\beta a^2}{D}\right)} \tag{7 b}$$

It will be observed that the unknown r has disappeared from (7 b) and the only unknowns are β and D; it is then enough to have a pair of two equations (7 b) and solving them we get β and D.

Once β and D are known from (7 b) only P_3 remains to be calculated before p_e is determined from (5). P_3, as stated earlier, $= p_{max}$. $\bar{p}_{T(t=o)}$ is experimentally determined; to this value must be added the difference $p_{max} - \bar{p}_{T(t=o)}$ in order to give p_{max} itself. By definition $p_{max} = \bar{p}_{e(t=o)}$. $\bar{p}_{T(t=o)}$ may also be derived from (2 a) and

$$\bar{p}_{T(t=o)} = \left[\frac{3}{4\pi a_3 \alpha} \int_o^a 4\pi r^2 \alpha(2a) dr\right]_{t=o} = p_{e(t=o)} - \frac{ma^2}{15\,D} \tag{16}$$

It follows that $p_{max} - \bar{p}_{T(t=o)} = \dfrac{ma^2}{15\,D}$ and therefore

$$P_3 = \bar{p}_{T(t=o)} + \frac{ma^2}{15\,D} \tag{17}$$

For the rest of the calculations the procedure is the same as for the ideal, pointlike electrode.

The practical application

In order to get sufficient data to show how the theory outlined above may be applied, some preliminary measurements were done with the following methods.

The arterial and the subcutaneous p_{O_2} were measured at the same time while a subject was given air or pure oxygen through a mouthpiece.

The arterial p_{O_2} was measured with the Clark electrode as described by ROOTH *et al.* (1959). A plastic catheter was inserted into the brachial artery and blood samples were drawn at known intervals. The dead space in the syringes was filled with heparin. The blood was drawn as quickly as possible and the sample considered as taken when half the syringe was filled. Because of the oxygen consumption of the blood itself, the observed p_{O_2} measurements were corrected for the time when the blood was drawn using the figures for the oxygen consumption of the whole blood given by DEWARDENER and YOUNG (1951).

The tissue p_{O_2} was measured by inserting a platinum electrode 0.2 mm in diameter about 2 mm into the subcutis of the forearm as a cathode, while a silver-silverchloride anode was placed on the skin a few cm. from the platinum cathode. 0.6 V was applied between the electrodes and the resulting current recorded by means of a LKB-Blomgren polarograph and a Leeds & Northrup Speedomax recorder. The platinum electrode was of the same type as that used by MONTGOMERY and HORWITZ but in addition it was covered with a thin film of plastic, as suggested by BARTELS *et al.* (1959). The tip of the electrode was immersed in a solution of 0.2 % polystyrene in trichlorethylene and afterwards the electrode was dried for six hours at 60° C. This covering of the electrode made it much more stable and also made the results become more reproducible. If bleeding occurred during the insertion of the electrode this was replaced in another hole.

The calibration of the platinum electrode was made in a Ringer solution of known p_{O_2}. The readings were not appreciably affected by stirring the solution and the same calibration was found valid for blood plasma. Recordings obtained in several healthy subjects breathing air gave values, calculated by this calibration, of about 40 mm Hg, which is the value arrived at by the use of the subcutaneous bubble technique (CAMPBELL 1924—25). We therefore used this calibration of the covered electrode in spite of the difference obtained in different media by MONTGOMERY (1957) and by INCH (1958) using bare platinum electrodes. However, the calibration method is not ideal and it will be an advantage to the measurements once it is possible to obtain absolute readings.

In several experiments uncovered platinum electrodes were used and the time between the initiation of the oxygen breathing and the rise in the O_2 recording was the same as when the polystyrene covered electrode was used. The steepness of the curve was also similar. Therefore, the covering of the electrode does not seem to affect the response time of the electrode.

The measurements could also be improved by taking due account to the time lag in the response of the *Pt* electrode and of the recorder.

In order to be able to solve the equations, besides the data obtained from these p_{O_2} measurements, figures are needed for m and a. For an estimation of the oxygen consumption this was measured on a piece of subcutaneous tissue immediately after it was excised and was found to be $0.156 \cdot 10^{-7}$ g/sec/g tissue. This was measured polarographically with the dropping mercury electrode by analyzing the drop in p_{O_2} caused by the metabolism of a piece of tissue immersed in a glucose Ringer solution at 37° C.

WETZEL and ZOTTERMAN (1926) give a mean of 7 capillaries per mm in the subcutis of the forearm of man, which gives a value of 0.007 cm for a.

Because of the inaccuracy of the calibration of the electrodes and of the figures m and a and because no correction was made for the actual temperature at the point of p_{O_2} measurement in the tissue, the value of the experiments described lies in the possibility of applying the equations for a practical purpose and not in the physiological results obtained.

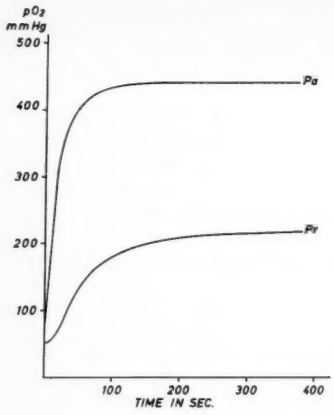

Fig. 4. The increase in oxygen tension in the artery and in the subcutaneous tissue during oxygen inhalation. Case 1.

Calculations

The changes in tissue and arterial oxygen pressure when a subject is switched over from breathing air to breathing pure oxygen is shown in Fig. 4. The constants P_1 and δ of equation (4) were calculated from the experimental findings by the method of trial and error.

For the calculation of the oxygen diffusion coefficient only the tissue curve is needed. A set of two equations (7 b) is established with the experimental p_T values read off from the tissue curve together with the corresponding values for t. The values chosen for t must be such that p_T is > 150 mm/Hg in order to avoid any interference from an inconstant γ caused by unsaturation of the hemoglobin.

When writing down the constants, meticulous care must be taken to give all the data in the same unitary system. The C. G. S. system was here adopted for the calculations, but the results are given in mm Hg.

It was found convenient for the sake of the calculations to rewrite equation (7 b) by introducing $x = \beta\, a^2/D$ after which it assumes the form

$$A - \frac{B}{x} e^{-\beta^2 t}\left[1 - \sqrt{x}\cot\sqrt{x}\right] + \frac{2x}{\pi^2}\sum_{n=1}^{\infty}\frac{e^{-n^2\pi^2 t\frac{\beta^2}{x}}}{n^2(n^2\pi^2 - x)} = O \qquad (7\,c)$$

where A and B are known figures.

Two limits were chosen for x so as to include all the physiologically possible values for D and within these limits the equations were solved. (D for the tissues should be lower than that of water which is $1.8 \cdot 10^{-5}$). Also, $\beta \leqslant \delta$ because the tissue p_{O_2} cannot increase as rapidly as the arterial p_{O_2} which furnishes the increase to the tissue.

In a diagram β/x, two point by point lines are plotted, each one representing the solution of one of the two (7 c) equations. The points of intersection of the two lines are the solutions of the system. If several solutions are obtained each one will have to be tried. It will then be found that only one single solution gives satisfactory figures such as positive values for the blood flow.

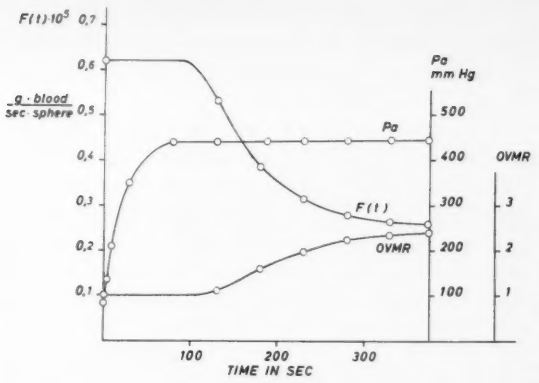

Fig. 5. The relation between arterial oxygen tension, the capillary blood flow and oxygen vasomotor reaction during oxygen inhalation. Case 1.

In order to reduce the time for solving a set of equations (7 c), it was found convenient to tabulate the terms $\frac{1}{x}\left[1 - \sqrt{x}\cot\sqrt{x}\right]$ and $\frac{2x}{\pi^2}\,\frac{1}{\pi^2 - x}$ These tables may then be used for different sets of equations as the factors used are independent of the individual values of β and D.

Equations (7 c) may then be solved by the use of a common office calculator and a long slide rule.

When inserting m in (10) and (17) it must be observed that in (10) m is expressed as g O_2 consumed/sec./cc. tissue but in (17) it is expressed as a decrease in oxygen tension/sec.

$\partial Q_1/\partial t$ is calculated from (10) and $\partial Q_2/\partial t$ is calculated from (11). By adding $\partial Q_1/\partial t$ and $\partial Q_2/\partial t$ we get $\partial Q/\partial$t.

The capillary flow is calculated from (14) and in order to use this equation $p_{(a-\bar{c})}$ must be calculated. Now $p_{(a-\bar{c})}$ is given by (9) where all the figures are known except P_3 which is obtained from (17).

Finally the vasomotor reaction is calculated according to (15).

As stated above, the flow is only calculated from the time when the tissue pressure is above 150 mm/Hg, *i. e.* when $p_a > 300 - 400$ mm/Hg and the hemoglobin is totally saturated and therefore γ is constant. The initial flow may be calculated by introducing a γ valid for this time. This new γ^1 depends upon the oxygen dissociation curve. γ^1 actually consist of two parts: the original γ and γ_{Hb} which is calculated as follows.

It must be determined how much oxygen the arterial blood releases when passing through the capillaries. The initial p_a is known and the initial $p_{\bar{c}}$ is already calculated from (5). The pH and the oxygen capacity must be measured and by the means of the oxygen dissociation curve the amount of oxygen released is calculated. This amount, divided by the pressure drop between the artery and the capillary, gives the average γ_{Hb} in that range of pressure. Finally $\gamma' = \gamma + \gamma_{Hb}$ is introduced in (14).

Thus the initial flow is obtained. From this point to the point correspondig to $p_T =$ = 150 mm/Hg, the curve representing the flow was drawn by hand. The details of the curve from the beginning to $p_T = 150$ may, if necessary, be calculated with the procedure described.

Results

Taking oxygen curves from the subcutaneous tissue of the forearm of two healthy male subjects we found for case 1 values of $D = 0.90 \cdot 10^{-5}$ and case 2 $1.07 \cdot 10^{-5}$ cm.²/Sec.

These values are of the expected order of magnitude as compared with in vitro measurements in different tissues — THEWS and NIESEL (1958—59).

The arterial p_{O_2}, the capillary flow and the vasomotor reaction are shown together in Fig. 5 (case 1). Very similar results were obtained in case 2.

If the blood flow is recalculated in ml of blood per min. per 100 c. c. subcutaneous tissue, the figures are 17 for case 1 and 16 for case 2. This agrees with plethysmographic measurements such as those of FELDER *et al.* (1954).

Discussion

The proposed mathematical treatment of the p_{O_2} curves from the subcutaneous tissues includes several assumptions, two not mentioned earlier are the constancy of the intercapillary distance (a) and the oxygen consumption of the tissue (m). It is possible that for exact measurements the figure for a may have to be determined in each case. If, for instance, m increases with increasing p_{O_2}, the figures for the capillary blood flow would be higher and the figure for the vasomotor reaction would be lower. This may have to be studied for each particular tissue and individual investigated. It follows that if a or m are variable, further mathematical treatment will be necessary, but the preliminary figures obtained seem to indicate that the assumptions made in the present study are justified.

In the calculation of the oxygen diffusion coefficient the results obtained are more exact as m does not enter the equation.

The effect of oxygen inhalation itself is directly evident from Fig. 5. It will be seen that about 2 min. after the inhalation of oxygen has started, the capillary flow is reduced and the gradual reduction continues for about 2 min. after the increase in p_a has stopped. This is the effect of the vasomotor reaction working at the level of the arterioli. If the initial diameter of the arterioli is considered as 1, the final diameter is 0.80 in case 1 and 0.81 in case 2. In the brain the corresponding figures have been observed by THEWS (personal communication) to be 1 : 0.83.

The method here described for studying the factors governing the oxygen flow to the tissues is specially suited for the subcutaneous tissue, because of the low oxygen consumption in relation to the blood flow but it may be used for other structures such as muscles.

The main value of the method for analyses of the p_T curves here presented lies in the possibility of measuring the oxygen diffusion coefficient in vivo.

Secondly the analyses have indicated which factors influence p_T curves obtainable with a platinum electrode.

The figures for the blood flow, oxygen vasomotor reaction and praecapillary

constriction during oxygen breathing would be obtainable with other, already established, methods. But under experimental circumstances where one wants to measure the tissue oxygen tension, it must be an advantage to obtain values for blood flow and so on without the use of additional equipment except for analyses of the arterial oxygen tension.

At one stage in this work we tried to do the calculations by means of an electronic computer. These calculations were kindly performed at the Department of Mathematics by Prof. C. E. Fröberg. We also wish to thank those who have kindly read the manuscript and given important criticism especially Dr. G. Thews, Kiel. This study has been supported by a grant from the Association for the Aid of Crippled Children, New York, U. S. A.

References

Bartels, H., W. Reinhardt and H. Bürger, Einfache Platinelektrode mit Kunststofftauchüberzug zur O_2-Druckmessung im Blut. *Naturwissenschaften* 1959. *24.* 672—673.

Campbell, J. A., Changes in the tensions of CO_2 and O_2 in gases injected under the skin and into the abdominal cavity. *J. Physiol.* (Lond.) 1924—1925. *59.* 1—16.

Chance, B., Cellular oxygen requirements. *Fed. Proc.* 1957. *16.* 671—680.

Cobb, S. and F. Fremont-Smith, The cerebral circulation. XVI. Changes in the human retinal circulation and in the pressure of the cerebrospinal fluid during inhalation of a mixture of carbon dioxide and oxygen. *Arch. Neurol. Phychiat.*, (Chicago) 1931. *26.* 731—736.

Crank, I., The Mathematics of Diffusion. *Lond. The Clarendon Press.* 1956. pp. 87.

Cusick, P. L., O. O. Benson and W. M. Boothby, Effect of anoxia and of high concentrations of oxygen on the retinal vessels: preliminary report. *Proc. Mayo Clin.* 1940. *15.* 500—502.

Felder, D., E. Russ, H. Montgomery and O. Horwitz, Relationship in the toe of skin surface temperature to mean blood flow measured with a plethysmograph. *Clin. Sci.* 1954. *13.* 251—257.

Fishman, A. P., M. H. Maxwell, C. H. Crowder and P. Morales, Kidney Function in Cor Pulmonale. Particular Consideration of Changes in Renal Hemodynamics and Sodium Excretion during Variation in Level of Oxygenation. *Circulation* 1951. *3.* 703—721.

Inch, W. R., Problems associated with the use of the exposed platinum electrode for measuring oxygen tension in vivo. *Canad. J. Biochem.* 1958. *36.* 1009—1021.

Krogh, A. J., The rate of diffusion of gases through animal tissues with some remarks on the coefficient of invasion. *J. Physiol.* (Lond.) 1919. *52.* 391—408.

Montgomery, H. and O. Horwitz, Oxygen tension of tissues by the polarographic method. 1. Introduction: oxygen tension and blood flow of the skin of human extremities. *J. clin. Invest.* 1950. *29.* 1120—1130.

Montgomery, H., Oxygen Tension of Tissues in Vivo. *Circulation*, 1957. *15.* 646—660.

Roughton, F. J. W., Diffusion and chemical reaction velocity in cylindrical and spherical systems of physiological interest. *Proc. roy. Soc. B.* 1952. *140.* 203—229.

Rooth, G., S. Sjöstedt and F. Caligara, Oxygen tension measurements in whole blood with the Clark cell. *Clin. Sci.* 1959. *18.* 379—388.

Thews, G., Über die mathematische Behandlung physiologischer Diffusionsprozesse in zylinderförmigen Objekten. *Acta biotheor.* (Leiden) 1953. *10.* 105—138. Part III/IV.

Thews, G. and W. Niesel, Zur Theorie der Sauerstoffdiffusion im Erythrocyten. *Arch. ges. Psychol.* 1958—1959. *268.* 318—333.

Tinel, J., Regulation de la circulation cérébrale a l'inhalation d'oxygène. *C. R. Soc. Biol.* (Paris) 1927. *96.* 665.

deWardener, H. E. and I. M. Young, Oxygen consumption of polycythaemic blood in vitro with a note on the arterial oxygen saturation in primary polycythaemia. *Clin. Sci.* 1951. *10.* 497—510.

Wetzel, N. C. and Y. Zotterman, On differences in the vascular colouration of various regions of the normal human skin. *Heart* 1926. *13.* 357—369.

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Analysis of Prenatal Spinal Reflex Activity in Sheep

By

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Abstract

L. ÄNGGÅRD, R. BERGSTRÖM and C. G. BERNHARD. *Analysis of prenatal spinal reflex activity in sheep.* Acta physiol. scand. 1961. *53.* 128—136. — An electrophysiological analysis was made of the prenatal development of the reflex muscular response to stretch in 17 non-anaesthetized sheep fetuses (calculated gestational age 53—132 days) kept in placental contact with the decerebrate ewe. Electrical muscle activity recorded with needle electrodes in the gastrocnemius muscle in response to stretch of the muscle appeared around the 60th day and was shown to be of reflex order. Antagonistic inhibition and inhibition evoked by skin stimulation were not found until later on, there being a short period in which no signs of central inhibition were obtained. Decerebrate rigidity was not obtained until the end of the gestation period, the total length of which is about 150 days in sheep.

Introduction

In earlier investigations by *e. g.* CARMICHAEL on the guinea pig (1934) and by BARCROFT and BARRON on the sheep (see review 1939) the prenatal development of both spontaneous and reflex movements in response to different types of stimulation was studied by observing the movements. A closer study of the appearance and the development of different spinal reflex functions with electrophysiological methods during the prenatal period has, however, never been made with the exception of some preliminary observations made by BERNHARD, KAISER and KOLMODIN (1961) in connection with their study of induced cortical activity in sheep fetuses during the last part of the gestational period.

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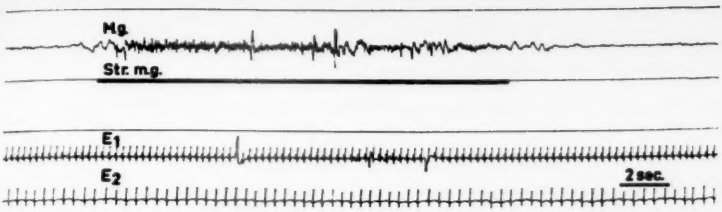

Fig. 1. Electromyogram from *m. gastrocnemius* (M. g.) in response to stretch (Str. m. g.) in a fetus with a calculated age of 61 days. Lower tracings show the electrocardiograms of fetus (E_1) and ewe (E_2).

This investigation aims at the elucidation of the prenatal development of segmental reflex activity in the lumbosacral part of the cord. Special attention was paid to the relation between spinal excitation and inhibition and their interrelation in the functional development of reflex differentiation.

A preliminary report was given at the 10th Scandinavian Congress of Physiology in Oslo (ÄNGGÅRD, BERGSTRÖM and BERNHARD 1960).

Methods

The experiments were performed on sheep fetuses with weights from 12 g to 2,650 g, the ages of which (53—132 days) were calculated from the equation given by HUGGETT and WIDDAS (1951). The ewe was decerebrated under thiogenal, anaesthetized with d-tubocurarine and kept on artificial ventilation (*cf.* BERNHARD, KAISER and KOLMODIN 1959). The fetus was delivered by caesarean section. Several fetuses with weights under 500 g were exteriorized in stages. Thus, the whole amniotic sac was first exposed, the spontaneous activity as well as certain reflexes and pulse rate of the fetus being observed under intra-amniotic conditions. In the next stage the fetus was exposed after rupturing the amniotic sac (see below). After exteriorization the fetus was mounted on a small table which was fixed close to the abdomen of the ewe and care was taken not to disturb the umbilical circulation. The fetus was kept in prone position on a bed of cotton wool and the preparation was covered with warm paraffin oil. One of the hind legs was fixed by means of needles through the cartilaginous tissue at the knee and ankle. The distal part of *m. gastrocnemius* was dissected free and by means of a thin thread attached to a lever by which the muscle could be passively stretched. In several preparations (see below) *m. tibialis anterior* was prepared in the same way. The action potentials from muscles were recorded with needle electrodes. The muscle activity as well as the ECG:s of the ewe and the fetus were recorded with a Grass electroencephalograph. The experiments usually started about 2 hours after the injection of thiogenal, the experiments thus being performed on non-anaesthetized preparations. In several fetuses (see below) decerebration was performed at the end of the experiment in order to study the appearance of decerebrate rigidity during the prenatal period.

Dextran was given continuously to the ewe by intravenous drip and care was taken to keep the whole preparation at body temperature.

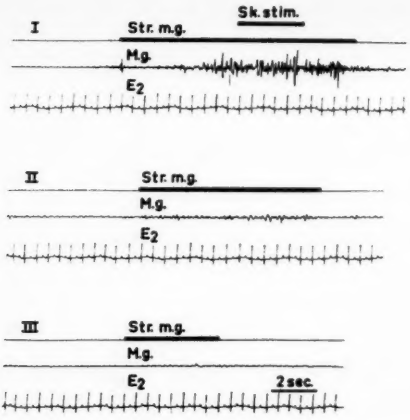

Fig. 2. Electromyogram from *m. gastrocnemius* (M. g.) in response to stretch (Str. m. g.) in a fetus with a calculated age of 60 days before spinal transection (I), after spinal transection at the upper lumbar level (II) and after spinal cord destruction (III). Sk. stim. marks skin stimulation of the foot ipsilateral to the muscle tested. Lower tracing (E_2) shows the ECG of the ewe.

Results

All fetuses used in this material showed spontaneous and reflex movements both when they were kept in the exposed unpunctured amniotic sac and after puncturing the sac. In the small fetuses the movements were, however, reduced and less frequent after emptying the amniotic sac. The fact that these movements again became more pronounced after refilling the sac with Ringer solution indicates that the reduction of the movements after emptying the sac is not due to deterioration of the preparation but depending on unfavourable mechanical conditions. The muscle contractions of the small fetuses are obviously too weak to result in movements when the fetus is not swimming freely in the surrounding intra-amniotic fluid. The ECG recordings showed that in general the pulse rate of the small fetuses was about 20 per cent higher when the fetus was totally exteriorized than when it was kept in the unpunctured amniotic sac. All the experiments presented in this material were performed on fetuses whose heart rate was of the same magnitude as that obtained shortly after delivery.

Fig. 1 shows the electrical muscular activity of *m. gastrocnemius* (M. g.) in response to a slight stretch of the muscle (marked by the thick line in the third tracing) in a 35 g fetus (calculated age 61 days). As seen there is an irregular muscle activity during the whole period of stretch. This activity often is of recruiting character during the first period of stretch. This is more clearly seen in Fig. 2 I, which shows the records from a fetus with a calculated age of 60 days. The activity was again recorded from the gastrocnemius muscle in response to stretch. In this experiment the spinal cord was then transected at the lower thoracic level and finally destroyed. The records in Fig. 2 II, taken after spinal transection, still show a response (M. g.) to stretch although the activity is now much less pronounced. After destruction of the lumbo-

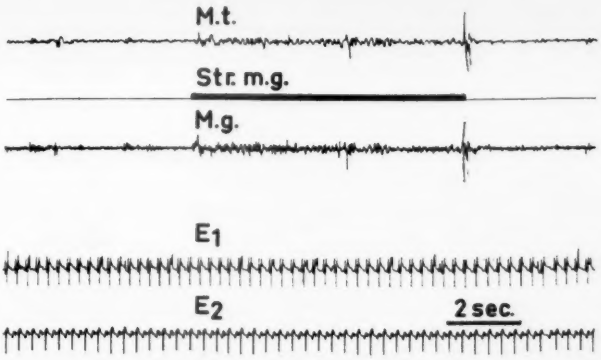

Fig. 3. Electromyogram from *m. tibialis anterior* (M. t.) and *m. gastrocnemius* (M. g.) in response to stretch of the latter (Str. m. g.) in a fetus with a calculated age of 67 days. Lower tracings show the ECG:s of the fetus (E_1)and the ewe (E_2).

sacral spinal cord the response to stretch disappeared totally, as seen in Fig. 2 III. Experiments of this type show that the muscular response to stretch is dependent on an intact spinal cord. As seen in Table I the muscular activity in *m. gastrocnemius* in response to stretch was tested in 17 fetuses of varying ages and was obtained in all fetuses older than 58 days, whereas no such response could be obtained in the younger fetuses tested (ages 53—58 days).

In 14 fetuses out of 17 preparations tested, the activity in *m. tibialis anterior* was recorded simultaneously. The upper and third tracings in Fig. 3 show the activity picked up by a needle electrode in *m. tibialis anterior* (M. t.) and *m. gastrocnemius* (M. g.), respectively, in a fetus having a calculated age of 67 days. As seen a stretch of the gastrocnemius muscle (Str. m. g.) is followed by activity not only in the gastrocnemius muscle but also in *m. tibialis anterior*. This type of response to stretch of *m. gastrocnemius* was obtained in all fetuses tested with ages between 60 and 81 days. In some of these fetuses stretching of the gastrocnemius muscle was performed also in periods when there was a background activity in *m. tibialis anterior* and vice versa. In these experiments signs of antagonistic inhibition were never seen but always signs of excitation of the antagonist, as illustrated in Fig. 3. The youngest fetus in which signs of antagonistic inhibition were observed had a calculated age of 90 days. An experiment on this fetus is illustrated in Fig. 4. The first record shows how the activity in *m. gastrocnemius* evoked by stretch (Str. m. g.) diminishes when a slight stretch is applied to *m. tibialis anterior* (Str. t.). The activity in *m. tibialis anterior* in response to stretch is shown in the third tracing (M. t.).

In most fetuses (15 out of 17) the effect of ipsilateral skin stimulation (pinching the skin of the foot) on the activity in *m. gastrocnemius* was investigated.

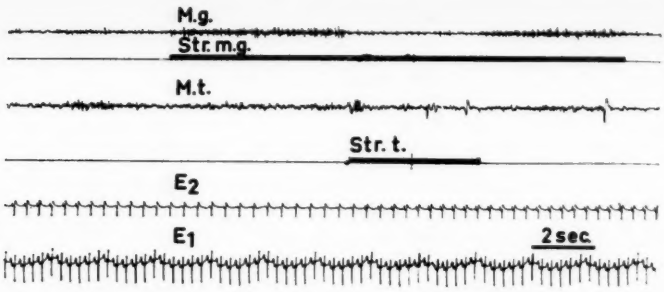

Fig. 4. Electromyogram from *m. gastrocnemius* (M. g.) and *m. tibialis anterior* (M. t.) in response to stretch of these muscles. (Str. m. g. and Str. t.) in a fetus with a calculated age of 90 days. E_1 and E_2 show the ECG:s of the fetus and the ewe respectively.

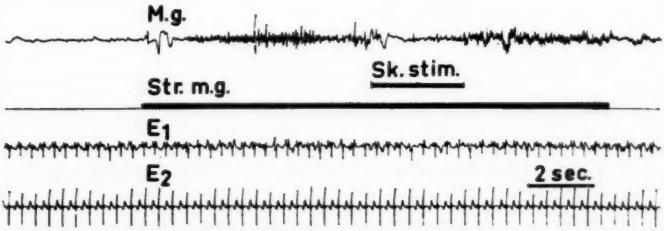

Fig. 5. Inhibition by ipsilateral skin stimulation (Sk. stim.) of the activity in *m. gastrocnemius* (M. g.) evoked by stretch of the muscle (Str. m. g.) in a fetus with a calculated age of 72 days. E_1 and E_2 show the ECG:s of the fetus and the ewe respectively.

In the experiment illustrated in Fig. 2 I, made on a 60-day fetus, ipsilateral skin stimulation (Sk. stim.) had no inhibitory effect on the activity in *m. gastrocnemius*. In the fetuses of this age skin stimulation was instead followed by an increase of activity. The earliest age at which ipsilateral skin stimulation was found to inhibit the activity in *m. gastrocnemius* was 72 days. This effect is illustrated in Fig. 5. The upper tracing shows the slowly increasing activity in *m. gastrocnemius* in response to stretch (Str. m. g.) which was inhibited by pinching the skin between the cloves on the same side (Sk. stim.). As seen in Table I, inhibition in response to ipsilateral skin stimulation was obtained in 7 fetuses out of 9 tested with ages above 69 days. Table I thus shows that whereas reflex excitation of the motoneurones evoked by stretching the muscle could be obtained around the 60th day, signs of classical inhibition (due to stretch of the antagonistic muscle or to ipsilateral skin stimulation) could not be obtained until later on during the prenatal period. Table I also shows that signs of reflex inhibition evoked either from antagonist or by skin stimulation could be demonstrated in 8 out of 10 fetuses tested with ages above 69 days.

Table I

Weight, g	Calculated age, days	Decerebrate rigidity	M. gast. response to stretch	Inhib. of M. gast. response by ipsilat. skin stim.	Inhib. of M. gast. response by stretch of M. tib. ant.
12	53	−	−	−	−
20	57	−	−	−	−
25	58		−	−	−
30	60	−	+	−	
35	61		+	−	−
90	69	−	+	−	−
126	72		+	+	−
250	81	−	+	+	−
440	90		+	−	+
500	92	−	+	+	−
885	103		+	−	−
1,570	117	−	+	+	−
1,670	119	−	+		
1,750	120	(+)	+	+	
1,850	122	−	+	+	−
2,300	127	−	+	+	+
2,650	132	+	+		−
	Newborn	+	+	+	+
	Adult	+	+	+	+

The effect of decerebration at different prenatal stages was also investigated. In this material 12 fetuses with varying calculated ages from 53 to 132 days were decerebrated. As mentioned above, decerebration was performed after the reflexes described above had been tested. Clear signs of decerebrate rigidity were only found at the end of the prenatal period (see Table I). In newborn lambs decerebrate rigidity was regularly obtained as in the adult sheep. In

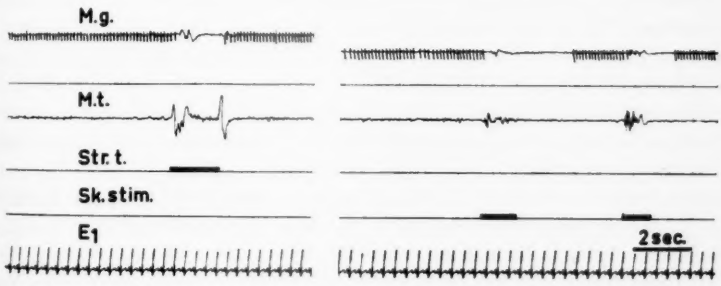

Fig. 6. Effect of ipsilateral skin stimulation (Sk. stim.) and stretch of *m. tibialis anterior* (Str. t.) on the electromyograms from *m. gastrocnemius* (M. g.) and *m. tibialis anterior* (M. t.) in a decerebrate preparation of a newborn lamb. E_1 shows the ECG.

some of the younger fetuses the different types of reflex activity described above were tested and found to be unchanged after decerebration.

Fig. 6 shows electromyograms from *m. gastrocnemius* and *m. tibialis anterior* in a decerebrate newborn lamb. The typical tonic discharge in the extensor muscle (M. g.) characteristic of the decerebrate state was never obtained in the fetuses before the end of the gestation period. Fig. 6 also shows the inhibition of the tonic discharge following a slight stretch of *m. tibialis anterior* (Str. t.), the reflex response of which is shown in the third tracing.

Discussion

It was found that muscle activity in response to stretch could not be evoked until about the 60th gestational day in the sheep, the total fetal period of which is about 150 days. It has been claimed that stretching a fetal muscle may act as a stimulus for the contraction of the fibres in the absence of the spinal cord (MINKOWSKI 1928). The fact that the muscular response described above was found to disappear after destruction of the lumbosacral part of the cord shows that this response to stretch is to be regarded as a spinal reflex. The question then arises if this "fetal myotatic reflex" which appears around the 60th day is evoked by stimulation of proprioceptors. In this context it should be pointed out that according to histological investigations (DICKSON 1940) simple definite spindles appear around this time in sheep. A comparison of our electrophysiological observations with these histological data would indicate that the "fetal myotatic reflex" demonstrated can be evoked from muscle spindles and that the lack of this type of reflex before the 60th day may be due to an incomplete development of this receptor function. So far we have not made latency measurements of the nerve or motor root reflex responses to electrical stimulation of afferent nerves at this early stage in order to obtain evidence for the existence of monosynaptic reflex transmission. In connection with other investigations (BERNHARD, KAISER and KOLMODIN 1959) such experiments were made on older fetuses which indicated the existence of a prenatal monosynaptic reflex transmission in sheep fetuses during the last part of the gestation period. In this context it should be mentioned that in newborn kittens proprioceptive reflexes are mediated by two-neuron arcs (SKOGLUND 1960 b).

At the fetal age of 60 days stretch is obviously followed by excitation not only of the motoneurones innervating the same muscle but also other muscles and even antagonists. Further, the experiments indicate that at this stage reflex excitation dominates since no signs of reflex inhibition following proprioceptive and exteroceptive stimulation could be found until the 72nd day. Thus, there seems to be a fetal period during which there is a pronounced lack of balance between the excitatory and inhibitory mechanisms which may explain the diffuse and widespread character of the reflex activity. The fact that during this period no signs of inhibition could be obtained as the result

of exteroceptive and proprioceptive stimulation, although both types of stimulation evoke widespread reflex movements, indicates that the absence of inhibition is of central order. This conclusion is in accordance with the results of spinal reflex experiments on newborn cats (MALCOLM 1953 and 1955), the functional central nervous maturation of which in relation to birth is delayed compared with the sheep. MALCOLM did not find any signs of spinal inhibition in the newborn cat. On the other hand, SKOGLUND who also investigated the postnatal development of spinal reflex activity in kittens found inhibition of extensors from flexors to be potent and long-lasting (1960 d) and referred the different results in newborn kittens to variations in maturity of the kittens at birth (1960 a, b and c).

It should be mentioned that muscular activity can be evoked by exteroceptive stimulation at earlier fetal ages (in sheep from about the 35th—40th day) which activity seems to be of reflex order, the trigeminal region being the most sensitive reflexogenic zone in the earliest stages (see BARCROFT and BARRON 1939). The response to stimulation of the skin, *e. g.* on the nose or on a leg in these young fetuses develops into a generalized reflex movement which according to BARCROFT and BARRON eventually becomes restricted to the muscles of the region stimulated. We have also studied this early generalized type of fetal reflex response to exteroceptive stimulation under intra-amniotic and extra-amniotic conditions. In general it was found that the generalized response pattern faded at the stage when the balance between excitation and inhibition appeared in the segmental reflex tested. According to the data given by BARCROFT and BARRON (1939), who only observed the movements following different types of stimulation, diffuse reflex responses to proprioceptive stimulation should appear somewhat earlier (48th day) than found in our electrophysiological investigations (60th day) but since they used flipping of the limb as stimulus, exteroceptive stimulation may have been involved in their experiments.

Finally, both the fetal myotatic response and peripherally evoked inhibition of this response were observed long before the appearance of decerebrate rigidity characterized by the sustained tonic muscle activity which was obtained in the last part of the prenatal period and in the newborn lamb. It is interesting to note that in newborn kittens no tonic reflexes can be obtained from the muscles concerned with decerebrate rigidity (SKOGLUND 1960 a). In this animal, the discharge of receptors in the gastrocnemius muscle in response to stretch was found to be phasic at birth, according to recent investigations by SKOGLUND (1960 c) who also concludes that post-tetanic potentiation plays an essential role for the appearance of the tonic stretch reflex (1960 e).

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References

ÄNGGÅRD, L., R. BERGSTRÖM and C. G. BERNHARD, Analysis of some prenatal reflex functions in sheep. *Acta physiol. scand.* 1960. *50.* Suppl. 175. 23—24.

BARCROFT, J. and D. H. BARRON, Movement in the mammalian foetus. *Ergebn. Physiol.* 1939. *42.* 107—152.

BERNHARD, C. G., I. H. KAISER and G. M. KOLMODIN, On the development of cortical activity in fetal sheep. *Acta physiol. scand.* 1959. *47.* 333—349.

BERNHARD, C. G., I. H. KAISER and G. M. KOLMODIN, Investigations on the epileptogenic properties of the developing brain in the fetal sheep. To be published. 1961.

CARMICHAEL, L., An experimental study in the prenatal guinea-pig of the origin and development of reflexes and patterns of behavior in relation to the stimulation of specific receptor areas during the period of active foetal life. *Genet. Psychol. Monogr.* 1934. *16.* 337—349.

DICKSON, L. M., The development of nerve-endings in the respiratory muscles of the sheep. *J. Anat.* (Lond.) 1939/40. *74.* 268—276.

HUGGETT, A. ST. G. and W. F. WIDDAS, The relationship between mammalian foetal weight and conception age. *J. Physiol.* (Lond.) 1951. *114.* 306—317.

MALCOLM, J. L., Some observations on dorsal root potentials. The spinal cord. Ciba Found. Symp. *J.* & *A. Churchill Ltd.* London 1953. Pp. 84—91.

MALCOLM, J. L., The appearance of inhibition in the developing spinal cord of kittens. Biochemistry of the developing nervous system. *Academic Press Inc.* New York 1955. pp. 104—109.

MINKOWSKI, M., Neurobiologische Studien am menschlichen Foetus. *Handb. biol. Arb.Meth.* 1928. *5 B.* 511—618.

SKOGLUND, S., On the postnatal development of postural mechanisms as revealed by electromyography and myography in decerebrate kittens. *Acta physiol. scand.* 1960 a. *49.* 299—317.

SKOGLUND, S., The spinal transmission of proprioceptive reflexes and the postnatal development of conduction velocity in different hindlimb nerves in the kitten. *Acta physiol. scand.* 1960 b. *49.* 318—329.

SKOGLUND, S., The activity of muscle receptors in the kitten. *Acta physiol. scand.* 1960 c. *50.* 203—221.

SKOGLUND, S., Central connections and functions of muscle nerves in the kitten. *Acta physiol. scand.* 1960 d. *50.* 222—237.

SKOGLUND, S., The reactions to tetanic stimulation of the two-neuron arc in the kitten. *Acta physiol. scand.* 1960 e. *50.* 238—253.

Acta physiol. scand. 1961. 53. 137—141

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Myocardial Lactate Concentration in Guinea-Pigs, Normothermic and Hypothermic, and Hedgehogs, in a Hibernating and a Non-Hibernating State

By

ARNE HANSON and BENGT W. JOHANSSON

Received 28 April 1961

Abstract

HANSON, A. and B. W. JOHANSSON. *Myocardial lactate concentration in guinea-pigs, normothermic and hypothermic and hedgehogs, in a hibernating and a non-hibernating state.* Acta physiol. scand. 1961. *53.* 137—141. — No significant differences were observed in the lactate content of hearts from hibernating and non-hibernating hedgehogs in February-March and June-August, respectively. The lactate content of hearts from guinea-pigs at 38° C was somewhat lower than corresponding values obtained from hearts removed at 30° C but the significance was borderline. When the guinea-pigs were cooled to still lower temperatures there was no further increase of the lactate content, the result was rather the opposite; nor was any increase observed when the animals were kept at 30° C for 2 hours. Artificial respiration before the animals reached a temperature of 30° C had no effect on the lactate content. It is therefore concluded that these results do not speak in favor of the view that the electrocardiographic ST-T changes occurring in hypothermic non-hibernators are caused by a state of anoxia in the hypothermic organisms studied.

Since hypothermia became widely used in surgical practice in the beginning of the fifties a great many physiological investigations on this subject have been published. In spite of this many problems remain unsolved. One of these questions without definite answer concerns the etiology of the ST-T changes appearing in the electrocardiogram of homiothermic animals during hypothermia, changes that do not appear in the electrocardiogram of a hibernator (BIÖRCK and JOHANSSON 1955) Anoxia is one of the factors to which these changes have been attributed (LANGE, WEINER and GOLD 1949). To study this particular

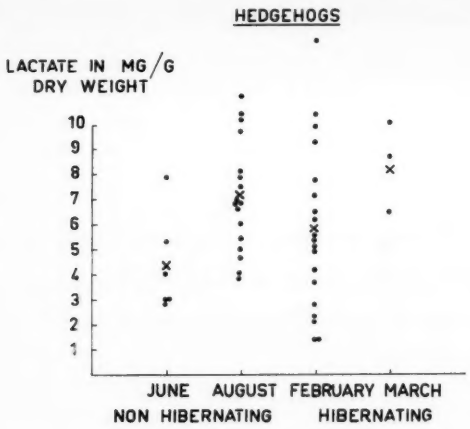

Fig 1. shows the myocardial lactate concentration in hedgehogs.

aspect the authors have determined the lactate concentration in myocardial tissue in a non-hibernator, the guinea-pig, at normal body temperature and during hypothermia and in a hibernator, the hedgehog, during the summer when the animals were not hibernating and during hibernation in the winter.

Material and methods

For the experiments we used 45 hedgehogs (Fig. 1) — 23 in a non-hibernating state of which 6 in June and 17 in August, and 22 in a hibernating state of which 19 in February and 3 in March — and 49 guinea-pigs (Fig. 2). Of the guinea-pigs 10 were used as controls; 15 were cooled to 30° C, 4 of which were kept at 30° C for two hours; 7 animals were also cooled to 30° C but in contrast to the preceding group of 15 guinea-pigs these animals received artificial respiration from a temperature of 32—34° C; 3 animals were cooled to 25° C and kept at this temperature for two hours receiving artificial respiration during the whole experiment; 11 animals were cooled to 20° C receiving artificial respiration from a temperature of 32°—34° C; 3 animals were cooled while receiving artificial respiration and then at 30° C the aorta and both venae cavae were clamped for 8 min. The hedgehogs were decapitated when hibernating while the non-hibernating animals were first anesthetized with nembutal. During cooling the guinea-pigs were also sufficiently anesthetized with nembutal as to inhibit shivering. The artificial respiration was performed with pure oxygen through a cannula in the trachea.

An enzymatic method described by Hohorst (1957) and slightly modified bu us was used for the lactate determinations. At the end of each experiment the heart was immediately removed, rinsed in cold water for a few seconds then quickly frozen by placing it in a small glass tube which was surrounded with solid carbon dioxide.

The lactate concentration was determined on wet tissue. The values were expressed as mg/g dry weight. To obtain the relation between dry and wet weights specimens of about 0.5 g tissue were dried at a temperature of 105° C for 2 hours and then kept in a vacuum desiccator with phosphorous pentoxide over night.

The t-analysis test was used for calculating possible differences between the series. A p value of 0.01 or less is regarded as significant.

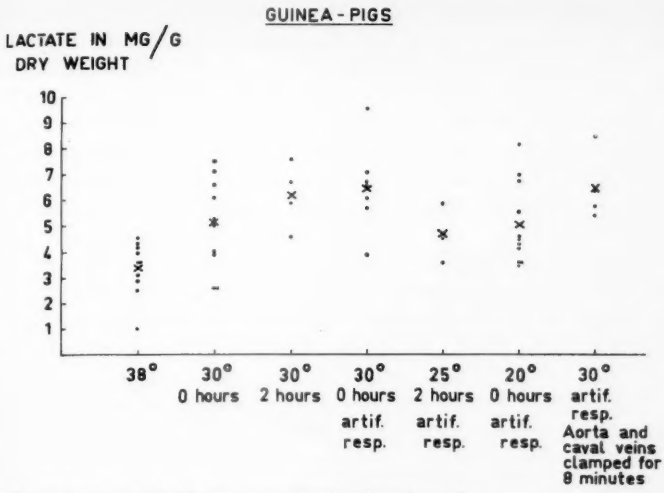

Fig 2. shows the myocardial lactate concentration in guinea-pigs.

Results

The results are given in Fig. 1 and 2. The range of the values obtained in the *hedgehogs* was rather wide especially in animals hibernating in February with values varying from 1.4 to 10.4 mg/g dry weight. No significant differences were found between the separate series.

There was a lower lactate concentration in the myocardial tissue from the control *guinea-pigs* in which the samples were taken just after anesthesia at normal body temperature than in the hypothermic animals ($0.01 < p < 0.02$). Animals kept for 2 hours at a body temperature of 30° C showed no significant difference from samples taken from animals that were killed immediately after they had reached 30° C. Nor was there any distinct difference ($p = 0.02$) between animals that had received artificial respiration to 30° C and those who had not. When the temperature was decreased further the lactate values did not change but remained on about the same level as at 30° C. The value obtained in animals in which the circulation had been clamped for 8 min was significantly higher than the control value ($0.001 < p < 0.01$) and also higher than the value obtained at 20° C ($p = 0.01$).

Discussion

This study was made to try to establish whether there is a difference in the metabolism between hibernators during the hibernating and non-hibernating period — some facts speak in favor of this — and to see how a non-hibernator reacts metabolically to a lowered body temperature. It is obvious from Fig. 1

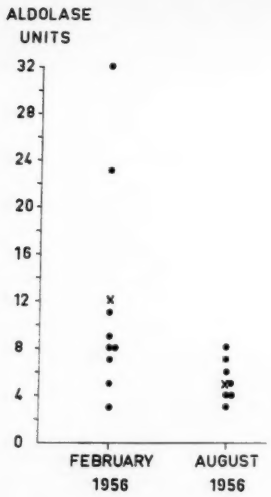

Fig. 3. shows the serum aldolase concentration in hedgehogs in a hibernating state in February and in a non-hibernating state in August.

that as far as lactate is concerned there were no great changes in the hibernators. We also studied the serum aldolase concentration in hibernating (February) and non-hibernating (August) hedgehogs.[1] As shown in Fig. 3 the mean value during hibernation was higher than that obtained in the summer. There are, however, rather few values and two in the February series are unusually high. If these two values are omitted and if one takes into consideration the higher hematocrit values in the hibernating animals (BIÖRCK, JOHANSSON and VEIGE 1956) it is doubtful whether there is any profound difference in serum aldolase concentration in hibernating and non-hibernating hedgehogs.

The lactate content is considered a reliable indicator of the degree of anaerobic metabolism in a tissue. As it has been claimed that hypothermia produces an anoxic state in the myocardium we thought it of interest to see if this possible anoxia was revealed in an increased lactate concentration in the tissues. Although the control values were lower than the values obtained at hypothermic levels this difference showed only a borderline significance. Even if the animals apparently breathed well at 30°C we administered artificial respiration to some of them (see Fig. 1). This, however, did not decrease the lactate level. When the animals were cooled to 20° C and 25° C and kept at these temperatures for 2 hours the lactate content did not increase. There was rather a contrary tendency when comparing with the values obtained at 30° C.

The concentration in blood of lactate during hypothermia has been reported by different authors. WADDELL, FAIRLY and BIGELOW (1957) obtained some

[1] Aldolase was chosen because it is one of the more important enzymes involved in the anaerobic metabolism of carbohydrates. Aldolase was determined according to a method by Sibley and Lehninger (1949).

elevation of blood lactate after the induction of anesthesia and hypothermia in man but no major changes occurred during cooling. In dogs KUHN *et al.* (1959) observed no change in the lactate concentration during cooling to 17° C. Both these teams found a marked increase of the lactate level after surgery and circulatory occlusion and following rewarming. THORN *et al.* (1958) found no change in lactate concentration in brains from rabbits at 37° C and 26° C, nor were any changes observed in other organs examined including the heart. However, if the brains were rendered anoxic or ischemic there occurred a marked increase in the lactate content.

Our results are in conformance with those published by THORN *et al.* (1958). The increase we noted at 30° C might be explained by a subclinical shivering, lactate proceeding into the myocardium as a result of the increased serum lactate concentration. It is not caused by anoxia due to insufficient respiration since artificial respiration with pure oxygen did not change the result. The hypothermia per se is probably not the cause of the increase (which shows only a borderline significance) for there is no further increase with lowering of the temperature.

The electrocardiographic changes observed during hypothermia have been interpreted as being of an anoxic nature (LANGE *et al.* 1949). The present investigation does not support this view since the increase in lactate concentration is only of marginal significance and does not increase with lowered temperature as do the electrocardiographic changes.

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We gratefully acknowledge the technical assistance by Miss ANNA-LENA SAMUELSSON.

References

BIÖRCK, G. and B. W. JOHANSSON, Comparative studies on temperature effects upon the electrocardiogram in some vertebrates. *Acta physiol. scand.* 1955. *34.* 257—272.

BIÖRCK, G., B. W. JOHANSSON and S. VEIGE, Some laboratory data on hedgehogs, hibernating and non-hibernating. *Acta physiol. scand.* 1956. *37.* 281—294.

HOHORST, H. J., Enzymatische Bestimmung von L (+) — Milchsäure. Biochem. Z. 1957. *328.* 509—521.

KUHN, E., K. SCHREIER, W. WÖRNER, K. SPOHN, E. KOLB, J. HEINZEL and R. KRATZERT, Stoffwechseluntersuchungen bei tiefer Hypothermie unter 20° und langdauerndem artefiziellem Kreislaufstillstand. Klin. Wschr. 1959. *37.* 651—654.

LANGE, K., D. WEINER and M. GOLD, Studies on the mechanism of cardiac injury in experimental hypothermia. *Ann. in. Med.* 1949. *31.* 989—1002.

SIBLEY, J. A. and A. L. LEHNINGER, Determination of aldolase in animal tissues. *J. biol. Chem.* 1949. *177.* 859—872.

THORN, W., H. SCHOLL, G. PFLEIDERER and B. MUELDENER, Stoffwechselvorgänge im Gehirn bei normaler und herabgesetzter Körpertemperatur unter ischämischer und anoxischer Belastung. *J. Neurochem.* 1958. *2.* 150—165.

WADDELL, W. G., H. B. FAIRLY and W. G. BIGELOW, Improved management of clinical hypothermia, based upon related biochemical studies. *Ann. Surg.* 1957. *146.* 542—562.

Acta physiol. scand. 1961. 53. 142—150

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Studies in Neuromuscular Transmission

I. Influence on Neuromuscular Transmission of Alkalosis and Acidosis

By

INGRID GAMSTORP[1] and ERIK VINNARS[2]

Received 4 May 1961

Abstract

GAMSTORP, I. and E. VINNARS. I. *Influence on neuromuscular transmission of alkalosis and acidosis.* Acta physiol. scand. 1961. *53.* 142—150. — The effect of alkalosis and acidosis on the neuromuscular transmission was studied in rabbits. The sciatic nerve was stimulated through bipolar electrode pairs and the electrical response of the gastrocnemius muscle was recorded. The stimulus necessary to evoke a maximal response, neuromuscular conduction time, nerve conduction time, amplitude and duration of response, refractory and irresponsive periods were measured as was the response to repeated stimulation at a rate of 3 per second. Respiratory alkalosis was produced by hyperventilation, respiratory acidosis by administration of 5, 10 or 20 % carbon dioxide in oxygen, metabolic alkalosis by i. v. injection of 0.5 N sodium carbonate and metabolic acidosis by i. v. injection of 0.5 N hydrochloric acid. A slight decrease in amplitude of the response was the only change noted during metabolic alkalosis. No significant alteration occurred during respiratory alkalosis or metabolic acidosis.

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Two changes were observed, occasionally after 5 and 10 % carbon dioxide, almost regularly after 20 % carbon dioxide, *i. e.* at pH below 7 and pCO_2 above 120 mm Hg: I. An increase in the stimulus necessary to evoke a maximal response. II. A slight prolongation of neuromuscular conduction time. These changes were not caused by release of adrenaline.

Changes in carbon dioxide tension and blood pH are known to influence the action of neuromuscular blocking agents in clinical anesthesia (DUNDEE 1952, SCURR 1954, GRAY and FENTON 1954). PAYNE (1958, 1960) and JOHANSEN and OSGOOD (1960) examined this influence experimentally in cats by recording the mechanical muscle response to nerve stimulation. With the same technique PAYNE (1958, 1960) also studied the effect of changes in carbon dioxide tension and blood pH on neuromuscular transmission in cats which received no neuromuscular blocking agents with variable results. The following experiments were designed to obtain further information on the effect of alkalosis and acidosis on neuromuscular transmission by employing a more precise technique of measurement.

Material and Methods

Thirty-five albino rabbits weighing 2.0 to 4.1 kg were used. Fourteen (group A) were exposed to respiratory alkalosis and acidosis, 7 (group B), used as a control study of group A, were given epinephrine i. v., and 14 (group C) were exposed to metabolic alkalosis and acidosis. Pentobarbital sodium (28 mg per kg) was given i. v. for the operative procedure about 2 hours before the experiment started. Procaine chloride locally was the only other anesthetic administered. The right femoral artery and vein were catheterized. A tracheal cannula was inserted through a tracheostomy in the rabbits belonging to group A to facilitate hyperventilation and the administration of carbon dioxide. The left sciatic nerve was exposed along its entire length and crushed and ligated as far proximal as possible. Bipolar stainless steel electrodes were used with the cathode placed most distally, the distance between anode and cathode being 5 mm. Two equal pairs of electrodes were applied to the nerve 3 to 6 cm apart, one close to the muscle and the other as far proximal as possible. The electrodes were sutured in place to prevent sliding along the nerve. The divided gluteal muscles were sutured and the skin closed. The rabbit was strapped to a table of appropriate size and the left leg was secured with adhesive tape.

The stimulus was a square wave pulse of 10 to 50 μsec duration. The voltage was increased until a maximal response was obtained and then increased about 25 % above this value (15—150 V). At this point it was always ascertained that a prolongation of the stimulus did not raise the amplitude of the response. The stimulus was maintained supramaximal and the necessary duration and voltage were recorded throughout the experiment. When double independent impulses with a variable interval were used, the second stimulus was about 25 % stronger than the first.

The electrical response in the left gastrocnemius muscle was recorded through a concentric needle electrode on a Disa electromyograph. The permanent recording of interrupted sweeps was made on photographic paper with a sweep speed of 2 m per sec. The stimulator triggered the sweep of the electromyograph thus making it possible to get the stimulus artefact and the response on the same record. At the start of the experiment, the needle electrode was adjusted until a simple response was obtained with

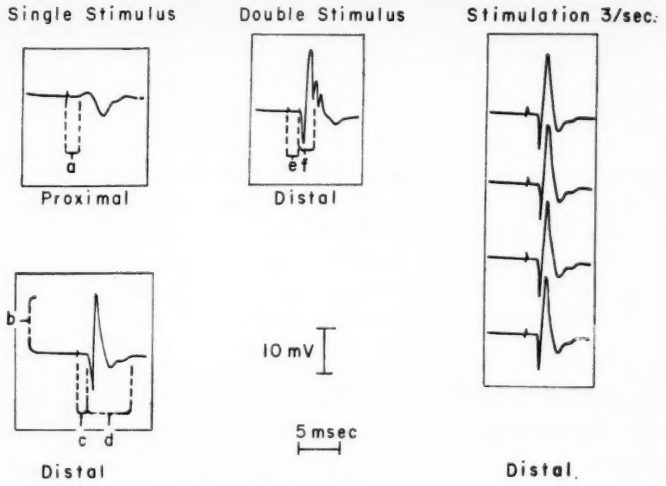

Fig. 1. Rabbit no. 37. Electrical response from 1. gastrocnemius muscle after supramaximal stimulation of sciatic nerve. Initial recording. c = neuromuscular conduction time. a—c = nerve conduction time. b = amplitude of response. d = duration of response. e = abosolute refractory period. f = absolute irresponsive period.

the highest possible amplitude and a thin, sharp initial deflection. The electrode was then fixed in this position with collodium and adhesive tape.

The following parameters were measured (Fig. 1):

1. The voltage and duration of the stimulus needed to give a maximal response. As a slight increase of the stimulus was occasionally necessary during prolonged experiments, the needed change in voltage had to be at least 20 V, with unaltered duration, to be considered significant.

2. Neuromuscular conduction time: the time interval from the beginning of the stimulus applied through the distal electrode pair to the beginning of the response (Fig. 1 c). Smaller changes than 0.2 msec could not be measured accurately.

3. Nerve conduction time: the time interval from the beginning of the stimulus applied through the proximal electrode pair to the beginning of the response minus the neuromuscular conduction time (Fig. 1 a—c). Smaller changes than 0.2 msec could not be measured accurately.

4. The amplitude and duration of the response (Fig. 1 b and d). For no apparent reason the amplitude of the potential could change 0.5—1 mV without an alteration of the shape of the response. If an obvious variation of the shape of the response occurred, indicating a shift in the position of the electrode, the experiment was discarded. Changes in the duration smaller than 0.5 msec could not be measured accurately.

5. The absolute refractory period: the shortest time interval between two supramaximal stimuli allowing both to evoke a response (Fig. 1 e). Smaller changes than 0.2 msec could not be measured accurately.

6. The absolute irresponsive period: the shortest time interval between the responses evoked by two supramaximal stimuli (Fig. 1 f). Smaller changes than 0.5 msec could not be measured accurately.

7. The response to repeated stimulation at a rate of 3 per sec. The amplitude of the response did not change during repeated stimulation unless a competitive neuromuscular block was produced.

In order to maintain a normal blood volume, dextran was infused i. v. in amounts roughly corresponding to the blood loss. Arterial blood pressure was measured constantly with an aneroid manometer connected to the catheter in the femoral artery and maintained at a level above 60 mm Hg. The intramuscular temperature was measured by a telethermometer. It varied between 41° and 45° C, not changing more than 1°.5 during a single experiment.

Respiratory alkalosis was produced by mechanical hyperventilation of the rabbit. The respirator used was a postive-negative pressure pump with which tidal volume was increased 2.5 to 3 times the level known to maintain a normal blood pH and carbon dioxide tension. Hyperventilation was continued for 10 min. Respiratory acidosis was produced by administration of 5, 10 and 20 % carbon dioxide in oxygen, each concentration for a period of 10 min. Normal ventilation with air for at least 10 min preceded the initial recordings. Arterial blood was drawn after each 10 min period.

Administration of carbon dioxide is known to cause a release of epinephrine (SECHZER *et al.* 1960) which might influence the neuromuscular transmission (GOFFART 1952, BOWMAN and ZAIMIS 1958). As the blood pressure usually showed a slight increase (from 90—100 to max. 120—130 mm Hg) during administration of 20 % carbon dioxide, the following experiments were performed on the rabbits in group B. Epinephrine (5 μg/ml) was injected i. v. for 10 min with a speed of 10 to 12 μg/min (= 3 to 5 μg/min/kg) which was considered to be above the amount released during severe stress (BOWMAN and ZAIMIS 1958). Recordings were made before, during and up to one hour after the injection. A few samples of arterial blood were drawn.

Metabolic alkalosis was produced by i.v. injection of 0.5 N sodium carbonate solution; 8 to 9 meq were given over a period of 2 to 3 min. Metabolic acidosis was produced by i. v. infusion of 0.5 N hydrochloric acid; 5 to 10 meq were given over a period of 2 to 4 min. In 2 experiments a second injection was given after completing the first one; a maximum of 13.5 meq was given. The total amount and the speed of the injection were adjusted so that a significant drop in blood pressure was avoided. Arterial blood was drawn before the injection, one minute after the end of the injection and 5 to 15 min later.

The blood samples were analyzed for carbon dioxide content by the method of van Slyke and the pH of the arterial blood was determined using a Beckman pH-meter. The partial pressure of carbon dioxide in arterial blood was calculated from the Singer-Hastings nomogram. The concentration of potassium in plasma was measured using a Beckman flamephotometer. To exclude the possible influence of the blood loss, 3 experiments with respiratory alkalosis, 5 with respiratory acidosis, 2 with infusion of epinephrine and 1 with metabolic alkalosis were performed without sampling of blood.

Results

Group A. Two severely anoxic rabbits were excluded from consideration. Results of analysis of blood samples from 5 of the animals with alkalosis and from 6 of the animals with acidosis showed that pure or almost pure respiratory alkalosis and acidosis were produced with no significant change in plasma potassium. During respiratory alkalosis the pH in the arterial blood was between 7.61 and 7.79 with the partial pressure of carbon dioxide between < 10 and 18 mm Hg. Corresponding values during respiratory acidosis were

after 5 % carbon dioxide 7.26—7.30 and 48—54 mm Hg, after 10 % carbon dioxide 7.02—7.20 and 62—92 mm Hg, and after 20 % carbon dioxide 6.83—6.98 with the carbon dioxide tension invariably above 120 mm Hg. All plasma potassium values were between 2.2 and 4.6 meq/l and the maximal change during one and the same experiment did not exceed 1.5 meq/l.

The results of the neuromuscular measurements are summarized under the headings defined in the section of methods.

Respiratory alkalosis.

1—7. No significant influence on the neuromuscular transmission was found in the 8 exp.

Respiratory acidosis.

1. The stimulus necessary to evoke a maximal response was found to be increased in 7 of 8 exp. after 20 % carbon dioxide, in 4 of 7 after 10 % carbon dioxide and in 4 of 7 after 5 % carbon dioxide. In 5 of 5 exp. the stimulus necessary to evoke a maximal response again decreased after normal ventilation with air. One rabbit was exposed to 20 % carbon dioxide for a second period of 10 min which again raised the stimulus necessary to evoke a maximal response.

2. The neuromuscular conduction time increased 0.2 to 0.6 msec in 9 of 11 exp. after 20 % carbon dioxide and did not change significantly in the other 2. In 4 of 4 exp. it returned to its initial value on normal ventilation with air. One rabbit was exposed to 20 % carbon dioxide for a second period of 10 min which again produced a 0.3 msec increase in neuromuscular conduction time. After 10 % carbon dioxide neuromuscular conduction time increased by as much as 0.2 msec in only 1 of 11 exp. and after 5 % carbon dioxide in only 1 of 9 exp. It did not change significantly in the other exp. (Fig. 1 and 2).

3. Nerve conduction time decreased 0.3 msec in one experiment and did not change significantly in the other 6. However, this figure being the small difference between 2 larger numbers is a less accurate measurement than the neuromuscular conduction time.

4. Provided the stimulus was maintained supramaximal by increasing it when necessary, a significant decrease in the amplitude of the response was recorded in 2 exp. only. An increase was never found. The duration of the response did not change.

5. No constant change in the absolute refractory period was found.

6. No constant change in the irresponsive period was found.

7. Subsequent responses to repeated stimulation at a rate of 3 per second showed no decrease in amplitude as compared with the first one.

The results of the experiments performed without sampling of blood did not differ from those in which blood was obtained.

Group B. Analysis of blood samples drawn before and immediately after injection of epinephrine in 5 exp. showed that no significant changes had occurred. The results of the neuromuscular measurements are summarized under the headings defined in the section of methods.

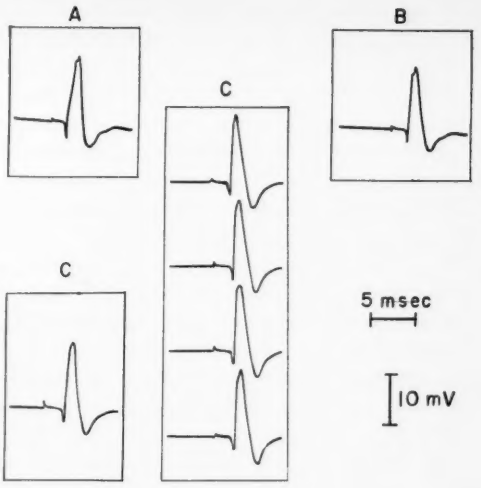

Fig. 2. Rabbit no. 37, same experiment as in Fig. 1; only distal stimulation.
A. Recording after administration of 5 % carbon dioxide for 10 min.
B. Recording after administration of 10 % carbon dioxide for 10 min.
C. Recording after administration of 20 % carbon dioxide for 10 min.
Lines on the illustrations have been darkened for photographic purposes.

1. The stimulus necessary to evoke a maximal response was found to be increased during the injection of epinephrine in 2 of 7 exp.

2—7. No significant influence on the neuromuscular transmission was found in the 7 exp.

Group C. During metabolic alkalosis the pH of the arterial blood increased to between 7.53 and 7.70 and the partial pressure of carbon dioxide was between 28.5 and 35 mm Hg (3 exp). The plasma potassium remained essentially unchanged. During metabolic acidosis the pH decreased to between 6.82 and 7.22. In 5 exp. it was below 7, in 2 7.00 and in 3 above 7. The partial pressure of carbon dioxide was between 21 and 39 mm Hg. In all of the experiments a slight increase (0.3—3 meq/l) of plasma potassium was found, the highest noted value being 6.5 meq/l, which is below the level necessary to induce changes in neuromuscular transmission (GAMSTORP and VINNARS, 1961).

The results of the neuromuscular measurements are summarized under the headings defined in the section of methods.

During metabolic alkalosis.

1. No significant change was noted in the stimulus necessary to evoke a maximal response.

2. Neuromuscular conduction time decreased 0.2 msec in 1 exp. and did not change in the other 3.

3. No change in nerve conduction time was found.

4. The amplitude of the response decreased 1 to 3 mV in all 4 exp. The duration of the response did not change.

5—7. No changes were found.

During metabolic acidosis.

1. The stimulus necessary to evoke a maximal response increased in 2 of 10 exp.

2. Neuromuscular conduction time increased with as much as 0.2 msec in only 1 of 10 exp.

3. Nerve conduction decreased 0.4 msec in 1 exp. and did not change in the other 9.

4. The amplitude of the response decreased 1 to 6 mV in 4 of 10 exp. The duration of the response did not change.

5. Absolute refractory period increased 0.3 msec in 1 of 10 exp. and did not change significantly in the other 9.

6. Absolute irresponsive period increased 1 msec in 1 of 10 exp. and did not change significantly in the other 9.

7. Subsequent responses to repeated stimulation at a rate of 3 per second showed no decrease in amplitude as compared with the first one.

Discussion

With the methods used respiratory alkalosis produced no measurable change in neuromuscular transmission. The only constant finding in metabolic alkalosis was a decrease in the amplitude of the response. Although slight, this change was noted in all the experiments. We have no explanation for this observation.

Neuromuscular transmission was altered in 2 ways during carbon dioxide inhalation. An increased stimulus was needed to evoke a maximal response and neuromuscular conduction time was prolonged. The first alteration occurred in all but one of the experiments in which the pH of the arterial blood was below 7 and carbon dioxide tension above 120 mm Hg and in half of those in which the acidosis was less severe and was found to be reversible. If the stimulus was maintained supramaximal no constant change in amplitude of the response was found. In his experiments on cats, PAYNE (1958) found the height of the muscle twitch to decrease during carbon dioxide inhalation. There are several possible explanations for this discrepancy. First, different species were used and secondly PAYNE recorded the mechanical and we the electrical response to nerve stimulation. PAYNE does not give the number of experiments upon which his conclusions are based. Finally, although the strength and duration of the stimulus is given, he does not state whether it was maintained supramaximal. Thus, PAYNE's results might be explained by the same increase in threshold to maximal stimulation as was found in our experiments. It is of interest that as early as 1895, WALLER described a change in the excitability of the nerve during exposure to carbon dioxide. On isolated mammalian nerve exposed to different concentrations of carbon dioxide, LEHMANN (1937) found nerve excitability to decrease during acidosis.

The results of the experiments with epinephrine show that although the release of epinephrine may play a role it is not the whole explanation for the increase in the stimulus needed to evoke a maximal response.

The second of the alterations in neuromuscular transmission occurring during administration of carbon dioxide, namely prolongation of the neuromuscular conduction time was found in 9 of 11 exp. in which the pH of the arterial blood was below 7 and the carbon dioxide tension above 120 mm Hg but in only one of those in which the acidosis was less severe. This was probably not due to a reduction of conduction velocity since conduction time in the nerve itself was found to be unchanged. Conduction time, like duration of the response, refractory and irresponsive periods, cannot be analyzed unless the electrical response is recorded. To our knowledge there are no data in the literature with which to compare our results. A prolongation of the neuromuscular conduction time is also found in hyperpotassemia (GAMSTORP and VINNARS 1961). However, this cannot be the responsible mechanism in our experiments, since little or no increase in plasma potassium occurred. Moreover, the other characteristic findings of hyperpotassemia: prolonged duration of the response and increased refractory and irresponsive periods (GAMSTORP and VINNARS 1961), were not found during administration of carbon dioxide. A release of epinephrine cannot explain the change in neuromuscular conduction time. The normal response to repeated stimulation excludes a block of the type produced by curare.

Administration of carbon dioxide characteristically causes a decrease in pH and an increase in carbon dioxide tension. In order to distinguish between those 2 factors, the series of experiments were carried out in which metabolic acidosis was produced. As changes in neuromuscular transmission were noted mainly during severe respiratory acidosis, our aim was to produce a metabolic acidosis with the pH of the arterial blood below 7. This was successfully done in 5 exp. and i 2 additional ones the pH decreased to 7. In no instance did the partial pressure of carbon dioxide in arterial blood increase significantly.

Measurable changes in neuromuscular transmission during metabolic acidosis were few and inconstant. In none of the 7 exp. with pH at or below 7 did the stimulus necessary to evoke a maximal response increase, in only one was a slight increase in neuromuscular conduction time noted, in 2 a drop in amplitude, in none a prolongation of refractory period and in one a prolongation of irresponsive period. On the other hand, in the 3 exp. with less severe acidosis, 2 showed an increase of the stimulus necessary to evoke a maximal response, 2 a prolongation of refractory period. These findings could not be related to changes in plasma potassium.

In hyperpotassemia, with or without secondary acidosis, the findings were a prolongation in neuromuscular conduction time, increases in duration of response, refractory and irresponsive periods and a decrease in amplitude; these changes were not only of regular occurrence but larger than those noted

in metabolic acidosis (GAMSTORP and VINNARS 1961). It can be concluded that the changes in neuromuscular transmission in potassium intoxication are related to hyperpotassemia and not to the drop in pH.

The statement in the literature that the pH of the surrounding fluid influences nerve excitability (GRAHAM and LORENTE de Nó 1938) appears to be based on experiments on isolated nerve fibers in which experiments the pH of the surrounding fluid was changed by altering its carbon dioxide concentration (LEHMANN 1937). In our experiments carbon dioxide administration appears to influence neuromuscular transmission more than does the same reduction of pH when produced without increasing carbon dioxide tension.

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References

BOWMAN, W. C. and E. ZAIMIS, The effects of adrenaline, noradrenaline and isoprenaline on skeletal muscle contractions in the cat. *J. Physiol.* (Lond.) 1958. *144.* 92—107.

DUNDEE, J. W., Influence on controlled respiration on dosage of thiopentone and d-tubocurarine chloride required for abdominal surgery. *Brit. med. J.* 1952. *2.* 893—896.

GAMSTORP, I. and E. VINNARS, Studies in neuromuscular transmission. II. Influence on neuromuscular transmission of hyperpotassemia. *Acta physiol. scand.* 1961. *53.* 151—159.

GOFFART, M., Recherches relatives à l'action de l'adrénaline sur le muscle strié mammifère. *Arch. int. Physiol.* 1952. *60.* 318—418.

GRAHAM, H. T. and R. LORENTE DE NÓ, Recovery of blood perfused mammalian nerves. *Amer. J. Physiol.* 1938. *123.* 326—340.

GRAY, T. C. and E. S. N. FENTON, Carbon dioxide retention simulating curarization. *Brit. med. J.* 1954. *1.* 820—820.

JOHANSEN, S. H. and P. F. OSGOOD, Influence of hypercarbia on the activity of neuromuscular blocking agents in the cat. Personal communication. 1960.

LEHMANN, J. E., Effect of changes in pH on action of mammalian A nerve fibers. *Amer. J. Physiol.* 1937. *118.* 600—612.

PAYNE, J. P., The influence of carbon dioxide on the neuromuscular blocking activity of relaxant drugs in the cat. *Brit. J. Anaesth.* 1958. *30.* 206—216.

PAYNE, J. P., The influence of changes in blood pH on the neuromuscular blocking properties of tubocurarine and dimethyl tubocurarine in the cat. *Acta anaesth. scand.* 1960. *4.* 83—90.

SCURR, C. F., Carbon dioxide retention simulating curarization. *Brit. med. J.* 1954. *1.* 565—566.

SECHZER, P. H., L. D. EGBERT, H. W. LINDE, D. Y. COOPER, R. D. DRIPPS and H. L. PRICE, Effect of CO_2 inhalation on arterial pressure, ECG, and plasma catecholamines and 17-OH corticosteroids in normal man. *J. appl. Physiol.* 1960. *15.* 454—457.

WALLER, A. D., The action of anaesthetics upon isolated nerve. *J. Physiol.* (Lond.) 1895. *18.* 45—47 P.

Acta physiol. scand. 1961. 53. 151—159

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Studies in Neuromuscular Transmission

II. Influence on Neuromuscular Transmission of Hyperpotassemia

By

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Abstract

GAMSTORP, I. and E. VINNARS. II. *Influence on neuromuscular transmission of hyperpotassemia.* Acta physiol. scand. 1961. *53.* 151—159. — The effect of acute potassium intoxication on the neuromuscular transmission was studied in rabbits. The sciatic nerve was stimulated through bipolar electrode pairs and the electrical response of the gastrocnemius muscle was recorded. The stimulus necessary to evoke a maximal response, neuromuscular conduction time, nerve conduction time, amplitude and duration of response, refractory and irresponsive periods were measured as was the response to repeated stimulation at a rate of 3 per second. Hyperpotassemia was produced by i. v. infusion (1/2—1 1/2 ml per min.) of isotonic solution of potassium chloride over 1/2—3 1/2 hours. The observed changes were: Prolonged neuromuscular conduction time, decreased amplitude and prolonged duration of response, prolonged refractory and irresponsive periods. These findings can be explained by a slowing of depolarization and repolarization near, at or distal to the neuromuscular junction. They were not caused by the metabolic acidosis nor by the hyponatremia which in some of the experiments accompanied the hyperpotassemia.

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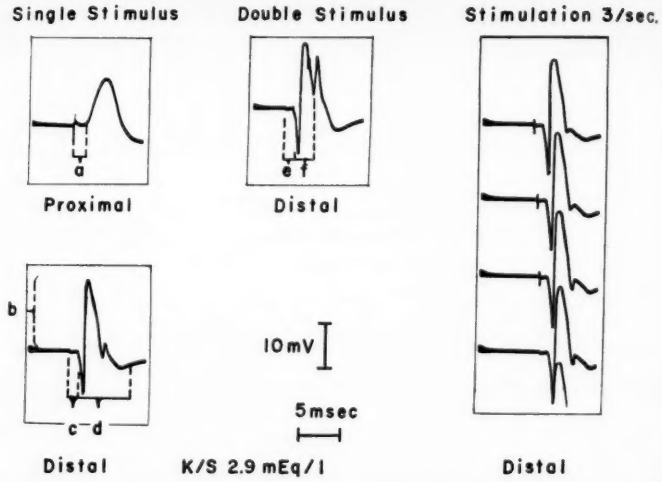

Fig. 1. Rabbit no. 90. Electrical response from 1. gastrocnemius muscle after supramaximal stimulation of sciatic nerve. Initial recording. c = neuromuscular conduction time. a—c = nerve conduction time. b = amplitude of response. d = duration of response. e = absolute refractory period. f = absolute irresponsive period.

For several decades it has been known that the potassium ion has a great influence on neuromuscular transmission (ASTOLFONI 1903). This has been studied experimentally in various species of animals (BROWN 1937, WALKER and LAPORTE 1947, WALKER 1948), including man (GROB, LILJESTRAND and JOHNS 1957). Clinical experience has also demonstrated that hyperpotassemia may cause hyperexcitability, twitching and weakness of muscles (FINCH and MARCHAND 1943, BULL, CARTER and LOWE 1953, BUCHTHAL, ENGBAEK and GAMSTORP 1958, MOLLARET, GOULON and TOURNILHAC 1958 a, b.) Electromyography has been performed only rarely in patients with hyperpotassemia (BUCHTHAL *et al.* 1958). The following experiments were dseigned to obtain more exact information about measurable changes in the neuromuscular transmission during acute potassium intoxication in the intact mammal.

Material and methods

Thirteen albino rabbits weighing 1.6 to 3.4 kg were used for the main experimental series. The method, briefly summarized here, has been described in detail elsewhere (GAMSTORP and VINNARS, 1961). Two equal pairs of electrodes were applied to the left sciatic nerve, one pair as far proximal as possible and one close to the muscle. The stimulus given through either of these electrode pairs was always supramaximal. The electrical response in the left gastrocnemius muscle was recorded through a concentric needle electrode on a Disa electromyograph. At the start of the experiment the needle electrode was adjusted until a simple response with a sharp, initial deflection was obtained; the electrode was then fixed in this position.

The following parameters were measured (see GAMSTORP and VINNARS 1961) (Fig. 1):

1. The voltage and duration of the stimulus needed to evoke a maximal response.
2. Neuromuscular conduction time: the time interval from the beginning of the stimulus applied through the distal electrode pair to the beginning of the response (Fig. 1, c).
3. Nerve conduction time: the time interval from the beginning of the stimulus applied through the proximal electrode pair to the beginning of the response minus neuromuscular conduction time (Fig. 1, a—c).
4. The amplitude and duration of the response (Fig. 1, b and d).
5. The absolute refractory period: the shortest time interval between 2 supramaximal stimuli allowing both to evoke a response (Fig. 1, e).
6. The absolute irresponsive period: the shortest time interval between the responses evoked by 2 supramaximal stimuli (Fig. 1, f).
7. The response to repeated stimulation at a rate of 3 per second.

Electrocardiogram was recorded on another channel through a needle electrode in the intercostal muscles; for this purpose the sweep speed was 0.2 m per second.

Arterial blood pressure remained at a level above 75 mm Hg. The intramuscular temperature varied between 42° and 45° C, not changing more than 0.8° during a single experiment.

Potassium intoxication was produced by intravenous infusion of an isotonic potassium chloride solution. During the infusion electrocardiogram and blood pressure were constantly watched. When a drop in the blood pressure or severe hyperpotassemic changes in the electrocardiogram developed the infusion was stopped until improvement occurred. Without these precautions sudden cardiac arrest occurred unexpectedly, leaving no time for recording neuromuscular transmission before convulsions and death.

The pH of the arterial blood was determined using a Beckman pH-meter and the concentration of potassium and sodium in plasma using a Beckman flamephotometer. One sample was always drawn before the experiment and one at the height of the intoxication; during several experiments intermediate samples were obtained.

As can be seen from Table I, a substantial drop in the concentration of plasma sodium occurred in several experiments. To exclude the possibility that a low concentration of plasma sodium might have influenced the results, another series of experiments was performed on 3 rabbits weighing 2.5 to 2.7 kg. The same operative procedure was done and the same parameters measured as previously described. These rabbits received intravenously 5 % dextrose in distilled water, containing enough potassium chloride to make its potassium concentration 5 meq/l.

Results

Three rabbits in the main series were excluded because of sudden cardiac arrest with convulsions and death preventing recording during potassium intoxication. The results in the remaining ones are summarized under the headings defined in the section of methods.

A. During potassium intoxication.

1. The stimulus necessary to evoke a maximal response increased in 2 of 10 exp.

2. Neuromuscular conduction time increased 0.3 to 1 msec in 8 of 10 exp., did not change significantly in one and was not measurable in one.

3. No significant change in nerve conduction time was found.

Table I. Blood values during potassium intoxication

Rabbit no.	Weight kg	Duration and amount of infusion	K meq/l	Na meq/l	pH
3	2.3	Before start of infusion	2.9	141	7.25
		60 min., 50 ml	5.8	135	7.32
		165 min., 130 ml	8.5	134	7.32
7	3.2	Before start of infusion	4.2	134	7.30
		45 min., 55 ml	10.7	130	7.25
9	2.2	Before start of infusion	3.1	136	7.30
		150 min., 90 ml	15.3	124	7.05
10	2.1	Before start of infusion	3.4	140	7.33
		45 min., 55 ml	4.3	136	7.25
		165 min., 80 ml	16.7	126	6.80
11	3.2	Before start of infusion	3.7	134	7.33
		60 min., 80 ml	7.6	127	7.30
		150 min., 140 ml	9.6	123	7.25
12	1.6	Before start of infusion	3.5	134	7.25
		100 min., 65 ml	7.4	126	7.25
		170 min., 95 ml	12.4	113	7.18
14	3.4	Before start of infusion	4.0	135	7.35
		75 min., 80 ml	7.6	124	7.30
		115 min., 105 ml	[1]7.2	128	7.30
27	3.7	Before start of infusion	2.4		
		35 min., 55 ml	[1]5.6		
31	2.4	Before start of infusion	3.8		
		110 min., 95 ml	5.7		
		210 min., 170 ml	9.2		
90	2.4	Before start of infusion	2.9	137	
		35 min., 50 ml	5.4	139	
		125 min., 100 ml	7.1	133	

[1] As the electrocardiogram showed severe hyperpotassemic changes, the possibility of a laboratory error has to be considered.

4. A decrease in the amplitude of the response was noted in all 10 exp.; the decrease varied from 1.2 to 14.6 mV. A prolongation of the response was also noted in all 10 exp.; the prolongation varied from 0.5 to 6 msec.

5. An increase in the absolute refractory period was noted in all 10 exp.; the increase varied from 0.3 to 1.7 msec.

6. An increase in the absolute irresponsive period was noted in all 10 exp.; the increase varied from 0.5 to 4.5 msec.

7. Subsequent responses to repeated stimulation at a rate of 3 per second showed no decrease in amplitude as compared with the first one.

Table II. Measurements performed simultaneously with the blood studies listed in Table I

Rabbit no.	Neuro-muscular cond. time msec.	Amplitude of response mV	Duration of response msec.	Abs. refractory period msec.	Abs. irresponsive period msec.
3	1.3	2.0	3.8	1.0	1.7
	1.3	1.5	3.8	0.9	1.7
	2.0	0.8	6.0	2.7	4.0
7	1.2	3.0	4.5	0.9	2.0
	1.6	1.5	5.0	1.5	2.5
9	1.2	7.3	3.5	1.6	2.2
	1.3	3.0	5.0	2.7	4.5
10	1.0	15	3.0	0.8	1.3
	1.0	17	4.0	0.7	2.5
	1.7	12.5	6.0	1.5	4.0
11	1.2	15	4.0	1.0	1.5
	1.5	10	4.0	1.5	3.0
	1.7	0.4	10.0	2.0	3.5
12	1.0	14	4.5	1.0	1.2
	1.1	1.6	6.0	1.0	2.0
	1.6	4.5	6.0	2.0	3.0
14	1.0	13	6.0	0.6	2.0
	1.2	12	6.0	1.4	4.5
	2.0	0.2	9.0	1.7	6.5
27	0.9	17	5.0	0.9	1.0
	1.2	7.0	7.0	1.9	2.7
31	—	17	6.0	0.7	1.6
	1.0	9.0	7.0	0.8	3.0
	1.2	5.5	7.0	1.6	4.5
90	0.9	17	7.0	1.0	2.2
	1.1	14	7.0	0.9	3.7
	1.2	11.5	8.0	1.3	4.4

B. During hyponatremia.

1—3. No significant changes were found.

4. The amplitude of the response decreased 2 to 4 mV in 2 of 3 exp. and did not change in one. The duration of the response decreased 0.5 msec in 1 exp., increased 1.0 msec in one and did not change in one.

5—7. No significant changes were found.

Blood values and pertinent measurements during potassium intoxication are summarized in Table I and II and during hyponatremia in Table III and IV. Samples of the recordings from 1 exp. are presented in Fig. 1 and 2.

Table III. Blood values during infusion of dextrose in water

Rabbit no.	Weight kg	Duration and amount of infusion	K meq/l	Na meq/l
87	2.7	Before start of infusion	3.0	139
		75 min., 200 ml	2.9	112
		120 min., 350 ml	3.0	104
88	2.5	Before start of infusion	2.6	138
		30 min., 200 ml	3.0	113
		50 min., 300 ml	3.9	106
89	2.7	Before start of infusion	3.2	145
		15 min., 100 ml	3.7	115
		54 min., 300 ml	3.8	101

Table IV. Measurements performed simultaneously with the blood studies listed in Table III

Rabbit no.	Neuro-muscular cond. time msec.	Amplitude of response mV	Duration of response msec.	Abs. refractory period msec.	Abs. irresponsive period msec.
87	1.4	17	6.0	0.7	2.3
	1.4	17	5.5	0.8	2.3
	1.5	17	5.5	0.6	2.3
88	1.6	14	5.0	1.5	3.7
	1.6	12	6.0	1.5	3.7
	1.5	13	6.0	1.3	3.7
89	1.3	15	5.5	0.9	1.2
	1.3	14	5.5	0.9	1.3
	1.3	11	5.5	1.0	1.6

Discussion

From clinical and experimental studies it is known that hyperpotassemia more often causes cardiac arrest than impaired function of skeletal muscles (FINCH and MARCHAND 1943, SMILLIE 1915, KOLFF 1950). The same was noted in our experiments. Only when ECG and blood pressure were constantly watched and the speed of the infusion adjusted to changes in them, was it possible to produce severe potassium intoxication without severely impaired circulation. On the other hand, the time for the potassium infusion was kept as short as possible. From Table I it is apparent that it never exceeded 3 1/2 hours and usually was about 2. As the uptake of potassium by skeletal muscles in the rabbit is known to be slow, this length of time will allow only a relatively small increase in muscle potassium to occur (FENN *et al.* 1941).

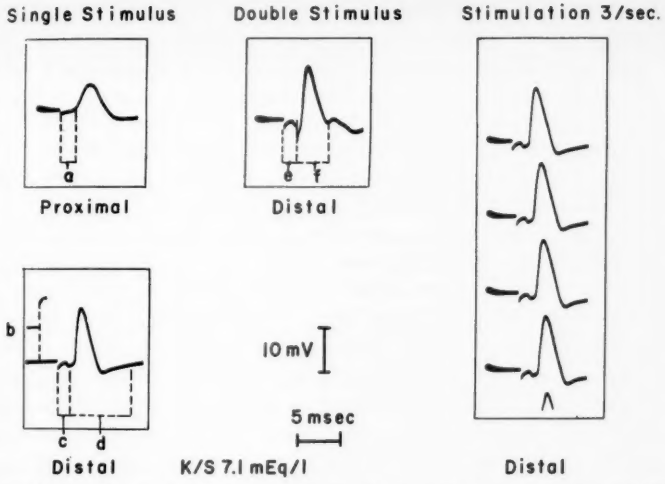

Fig. 2. Rabbit no. 90. Same experiment as in Fig. 1. Recording after intravenous infusion of 100 ml isotonic KCl solution over a period of 125 min. Same symbols as in Fig. 1. Lines on the illustrations have been darkened for photographic purposes.

As can be seen from Table I, infusion of potassium chloride caused 2 changes in the blood beside hyperpotassemia. First, a slight to moderate drop in sodium was noted. However, the same changes in neuromuscular transmission were found in experiments showing a small and a great decrease in sodium. Furthermore, no change in neuromuscular conduction time, refractory and irresponsive periods were noted during the experiments in which hyponatremia was produced. The low sodium may account for some of the decrease in amplitude of the response but can not explain the other findings observed during potassium intoxication.

Secondly, a metabolic acidosis was often produced. However, the same changes in neuromuscular transmission were noted in experiments showing severe acidosis as in those showing none. Furthermore, although it has been observed that neuromuscular conduction time is slightly prolonged in severe respiratory acidosis, neither this nor other changes in neuromuscular transmission were noted during severe metabolic acidosis (GAMSTORP and VINNARS 1961). The changes in neuromuscular transmission found during infusion of potassium chloride are thus related to the increase of potassium and not to a decrease in sodium and pH.

When directly applied to the muscle fiber, or given in "close arterial injection", potassium has a depolarizing effect (BUCHTHAL and LINHARD 1939). If the "close arterial injection" was given during intermittent supramaximal nerve stimulation the mechanical response of the muscle increased in amplitude

while the electrical one decreased (BROWN 1937). On recording mechanically and electrically the muscle response to nerve stimulation in rats during hyperpotassemia produced by an intraperitoneal injection of potassium chloride, WALKER (1948) was able to confirm both these observations concerning the amplitude. Furthermore, he found an increase in the interval between stimulus and response and in the duration of the response. He concluded that an excess of potassium delays both depolarization and repolarization and increases the duration of membrane breakdown.

Our experiments thus confirm to a large extent WALKER's observations and his interpretations. Neuromuscular conduction time was found to be increased while excitability and conduction velocity in the nerve itself were unchanged. This means that the delay occurs near, at or distal to the neuromuscular junction. A prolongation of the response may be caused by a slower conduction of the potential over the muscle fiber, by an increased asynchrony of the individual muscle fibers within a motor unit or by a slower repolarization. Hyperpotassemia of this degree has been shown not to decrease conduction velocity over the electrically stimulated muscle fiber (BUCHTHAL *et al.* 1958), and for this reason the first suggestion is improbable. An increased asynchrony will cause both lower amplitude and longer duration and can also explain the enhanced mechanical response found by others (BROWN 1937, BROWN and EULER 1938, WALKER 1948). Only a prolonged contraction time, *i. e.* a slower repolarization, however, can account for our findings of prolonged refractory and irresponsive periods. Thus, all of our findings in potassium intoxication can best be explained by a slower depolarization and repolarization near, at or distal to the neuromuscular junction.

We are indebted to Dr. PHILIP R. DODGE, Dr. ROBERT S. SCHWAB, and Dr. RAYMOND D. ADAMS, Neurology Service, and to Dr. JOHN D. CRAWFORD Children's Medical Service, for help and encouragement throughout the work and for placing equipment and laboratory facilities at our disposal.

References

ASTOLFONI, G., Richerche intorno all'azione farmacologica delle soluzioni dei sali di potassio. *Arch. int. Pharmacodyn.* 1903. *11.* 313—360.

BROWN, G. L., The action of potassium chloride on mammalian muscle. *J. Physiol.* (Lond.) 1937. *91.* 4—5 P.

BROWN, G. L. and U. S. VON EULER, After effects of tetanus on mammalian muscle. *J. Physiol.* (Lond.) 1938. *93.* 39—60.

BUCHTHAL, F. and J. LINHARD, Acetylcholine block of the motor endplate and electrical stimulation of nerve. *J. Physiol.* (Lond.) 1939. *95.* 59—60 P.

BUCHTHAL, F., L. ENGBAEK and I. GAMSTORP, Paresis and hyperexcitability in adynamia episodica hereditaria. *Neurology* 1958. *8.* 347—351.

BULL, G. M., A. B. CARTER and K. G. LOWE, Hyperpotassemic paralysis. *Lancet* 1953. *2.* 60—63.

FENN, W. O., T. R. NOONAN, L. J. MULLINS and L. HAEGE, Exchange of radioactive potassium with body potassium. *Amer. J. Physiol.* 1941. *135.* 149—163.

FINCH, C. A. and J. F. MARCHAND, Cardiac arrest by action of potassium. *Amer. J. med. Sci.* 1943. *206.* 507—520.

GAMSTORP, I. and E. VINNARS, Studies in neuromuscular transmission. I. Influence on neuromuscular transmission of alkalosis and acidosis. *Acta physiol. scand.* 1961. 142—150.

GROB, D., Å. LILJESTRAND and R. J. JOHNS, Potassium movement in normal subjects; effect on muscle function. *Amer. J. Med.* 1957. *23.* 340—355.

KOLFF, W. J., Serum potassium in uremia; report of 16 cases, some with paralysis. *J. Lab. clin. Med.* 1950. *36.* 719—728.

MOLLARET, P., M. GOULON and M. TOURNILHAC, Contribution à l'étude des paralysies avec hyperkaliémie. *Rev. neurol.* (Paris) 1958 a. *98.* 341—357.

MOLLARET, P., M. GOULON and M. TOURNILHAC, Contribution à l'étude des paralysies avec hyperkaliémie. *Rev. neurol.* (Paris) 1958 b. *99.* 241—263.

SMILLIE, W. G., Potassium intoxication in nephritis. *Arch. intern. Med.* 1915. *16.* 330—339.

WALKER, S. M. and Y. LAPORTE, Effects of potassium on endplate potential and neuromuscular transmission in curarized semitendinosus of frog. *J. Neurophysiol.* 1947. *10.* 79—85.

WALKER, S. M., Action potentials in rat muscle with twitch tension potentiated by KCl treatment, adrenalectomy, tetanus and treppe. *Amer. J. Physiol.* 1948. *154.* 63—72.

Acta physiol. scand. 1961. 53. 160—173

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Studies in Neuromuscular Transmission

III. Influence of Changes in Blood pH and Carbon Dioxide Tension on the Effect of Tubocurarine and Dimethyl Tubocurarine

By

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Abstract

GAMSTORP, I. and E. VINNARS. III. *Influence of changes in blood pH and carbon dioxide tension on the effect of tubocurarine and dimethyl tubocurarine.* Acta physiol. scand. 1961. *53.* 160—173. — The influence of changes in pH and the partial pressure of carbon dioxide in arterial blood on the neuromuscular blocking effect of a single dose of tubocurarine or dimethyl tubocurarine was investigated in rabbits. The sciatic nerve was stimulated supramaximally at a rate of 3 per second and the electrical response of the gastrocnemius muscle was recorded; the amplitude of the first and fourth response was measured. During the period of spontaneously subsiding block after a single injection of the blocking agent, a shift in the arterial pH was produced either with or without a change in carbon dioxide tension. An increase of the block during this period was always considered significant but a decrease of the block only if it was followed by a spontaenous increase. The action of tubocurarine increased during acidosis and diminished during alkalosis. This effect was greater during metabolic alkalosis and acidosis, corresponding to a more rapid shift in pH, than during respiratory alkalosis and acidosis, but no qualitative difference was observed. The effect of a shift in pH is explained by changes in the potency of tubocurarine. The action of dimethyl tubocurarine was not influenced by changes in pH or carbon dioxide tension.

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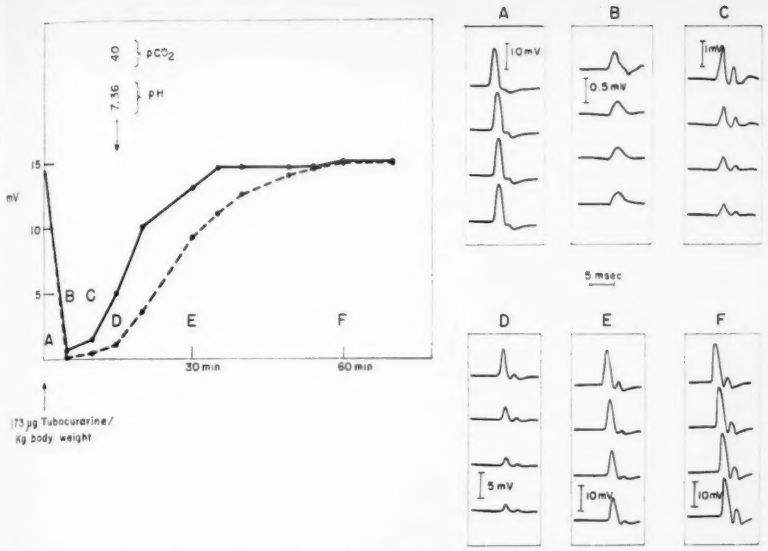

Fig. 1. Rabbit no. 61. The curve shows the amplitude of first response (unbroken line) and of fourth response (broken line), recorded electrically, in left gastrocnemius mucle after supramaximal stimulation of sciatic nerve at a rate of 3 per second. Examples of tracings are given to the right in the figure; letters indicate when the recordings were made.

Changes in carbon dioxide tension and blood pH are known to influence the action of neuromuscular blocking agents in clinical anesthesia (DUNDEE 1952, SCURR 1954, GRAY and FENTON 1954). PAYNE (1958, 1959) and JOHANSEN and OSGOOD (1960) have experimentally confirmed in cats the enhancing effect of carbon dioxide on the action of tubocurarine. The influence of carbon dioxide on the block caused by dimethyl tubocurarine was found to be in the opposite direction. Recently PAYNE (1960) found that the effect of both tubocurarine and dimethyl tubocurarine increased during metabolic alkalosis and decreased during metabolic acidosis. He concluded that carbon dioxide has a specific effect on tubocurarine differing from that of changes in pH. PAYNE's results and interpretation contrast with the observation of KALOW (1954) who found that a reversible change in the ionization of tubocurarine followed variations in pH and increased its blocking effect on the isolated nerve-muscle preparation when pH decreased. The following experiments were designed to obtain further information about variations in the sensitivity of the intact animal to tubocurarine and dimethyl tubocurarine.

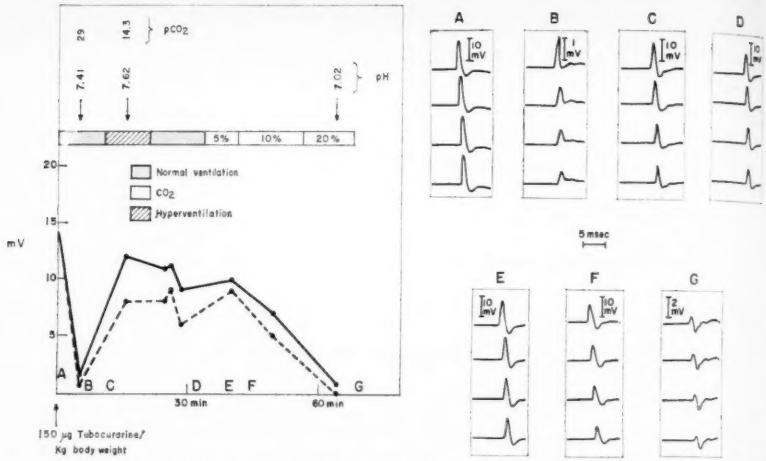

Fig. 2. Rabbit no. 32. The curve shows the amplitude of first response (unbroken line) and of fourth response (broken line), recorded electrically, in left gastrocnemius muscle after supramaximal stimulation of sciatic nerve at a rate of 3 per second. Type of ventilation, blood pH and pCO_2 are given above the curve. Examples of tracings are given to the right in the figure; letters indicate when the recordings were made.

Material and Methods

Forty-one albino rabbits weighing 2 to 4 kg were used. Twenty-one (group I) received tubocurarine and 20 (group II) dimethyl tubocurarine. For the operative procedure pentobarbital sodium (28 mg per kg) was given i. v. about 2 hours before the experiment started. Procaine chloride locally was the only other anesthetic administered. The right femoral artery and vein were catheterized. A tracheal cannula was inserted through a tracheostomy. The left sciatic nerve was exposed and a pair of stainless steel electrodes was applied to it. Proximal to the electrode pair the nerve was crushed and ligated.The divided gluteal muscles were sutured and the skin closed.

The stimulus was a square wave pulse of 10 to 50 μsec duration and 15 to 150 V strength. It was maintained supramaximal throughout the experiment. The electrical response of the left gastrocnemius muscle was recorded through a concentric needle electrode on a Disa electromyograph. The permanent recording of interrupted sweeps was made on a photographic paper with a sweep speed of 2 m per second. Stimulation at a rate of 3 per second for about 2 sec was used to demonstrate the effect of tubocurarine on the neuromuscular transmission; the amplitude of the first and fourth response was measured. At the start of the experiment the needle electrode was adjusted until a simple response was obtained with the highest possible amplitude and a sharp initial deflection. The electrode was then fixed in this position.

During curarization artificial respiration was given with a positive-negative pressure pump. Frequency and tidal volume were adjusted to maintain a normal blood pH and carbon dioxide tension except when alkalosis or acidosis was deliberately produced.

In order to maintain a normal blood volume dextran was infused i. v. in amounts roughly corresponding to the blood loss. Arterial blood pressure was measured con-

Table I. Blood values in respiratory alkalosis and acidosis

Rabbit no.	Tubocurarine dose mg/kg	Experimental condition	pH	pCO_2 mm Hg	CO_2 content meq/l	K meq/l
26	0.14	Normal ventilation	7.39	33	20.7	3.5
		Hyperventilation	7.58	18.6	17.7	3.5
		10 % CO_2	7.00	102.0	37.3	3.6
		20 % CO_2	6.87	121	24.9	
30	0.14	Normal ventilation	7.51	22	17.5	3.1
		Hyperventilation	7.79	10	14.2	3.4
		10 % CO_2	7.10	76	25.3	3.4
32	0.15	Normal ventilation	7.41	29	18.8	
		Hyperventilation	7.62	14.3	14.7	2.8
		10 % CO_2	7.41	37	24.1	4.1
		20 % CO_2	7.02			5.9
33	0.14	Hyperventilation	7.68	10	11.5	2.4
		Normal ventilation	7.43	20	13.6	2.1
		20 % CO_2	6.84	120	27.4	2.4
38	0.14	Hyperventilation	7.72	13.3	16.9	2.7
		Normal ventilation	7.46	24.2	17.4	2.6
		10 % CO_2	7.03	104	29.3	2.4
41	0.18	Hyperventilation	7.53	17.4	14.8	4.2
		Normal ventilation	7.29	30.2	15.2	3.4
		10 % CO_2	7.05	74	22.4	3.6

stantly and maintained at a level above 70 mm Hg. The intramuscular temperature varied between 41° and 45° C, not changing more than 1.5° during a single experiment.

Group I. An initial recording was made at the start of the experiment. A single dose of tubocurarine (0.14 to 0.18 mg per kg) was then given i. a. and a new recording was made 5 min later. During this time ventilation was maintained normal. In 5 rabbits (group 1 A) the effect of tubocurarine alone was followed until it disappeared, recordings being made every 5 min.

In 9 rabbits (group I B) alkalosis was induced by increasing tidal volume 2.5 to 3 times directly following the recording made 5 min after the injection of tubocurarine. Hyperventilation was continued for 10 min followed by 10 min of normal ventilation, after which acidosis was produced by administration of 5 (this concentration was tested in only 4 exp.), 10 and 20 % carbon dioxide in oxygen, each concentration for a period of 10 min.

At the end of each 10 min period a recording was regularly made and arterial blood was usually drawn. The pH of the arterial blood was determined using a Beckman pH-meter and carbon dioxide content was measured on the same sample by the method of van Slyke; the partial pressure of carbon dioxide in arterial blood was calculated from Singer-Hastings nomogram. The concentration of potassium in plasma was determined on a Beckman flamephotometer.

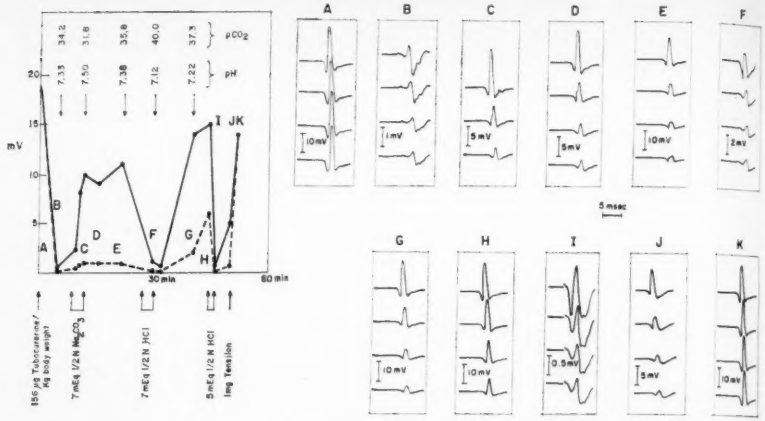

Fig. 3. Rabbit no. 47. The curve shows the amplitude of first response (unbroken line) and of fourth response (broken line), recorded electrically, in left gastrocnemius muscle after supramaximal stimulation of sciatic nerve at a rate of 3 per second. Type and amount of injection is given below the curve, blood pH and pCO_2 above it. Examples of tracings are given to the right in the figure; letters indicate when the recordings were made.

In 7 rabbits (group I C) alkalosis was produced by i. v. injection of 6 to 16 meq 0.5 N sodium carbonate solution over 2 to 3 min. The infusion started directly following the recording made 5 min after the injection of tubocurarine. Recordings were made within 1 min after the injection and about 5 to 10 min later. Within 15 min after the injection of sodium carbonate, metabolic acidosis was produced by i. v. injection of 7 to 9 meq 0.5 N hydrochloric acid over 2 to 5 min. During the injection the tidal volume was increased 10 to 20 % to maintain as normal a carbon dioxide tension as possible. Recordings were made within 1 min after the injection and 5 min after, and, if the rabbit survived long enough, 15 min later. Samples of blood for pH, carbon dioxide content and plasma potassium were obtained simultaneously with the electrical recordings.

Group II. An initial recording was made at the start of the experiment. A single dose of dimethyl tubocurarine (10 to 44 μg per kg) was given i. a. and a new recording was made 5 min later. In 4 rabbits (group II A) the effect of dimethyl tubocurarine alone was followed until it disappeared, recordings being made every 5 min.

In 6 rabbits (group II B) respiratory alkalosis and acidosis were induced in the same way as described for group I B. Four rabbits were first exposed to hyperventilation followed by normal ventilation and finally administration of carbon dioxide. In 2 rabbits the opposite order was used. Two rabbits were exposed twice to inhalation of 20 % carbon dioxide. At the end of each 10 min period a recording was made and usually arterial blood was drawn for determination of pH, carbon dioxide content and plasma potassium.

In 10 rabbits (group II C) metabolic alkalosis and acidosis were induced in the same way as described for group I C. In 6 rabbits alkalosis was first produced followed by acidosis, in 4 the opposite order was used.

As alkalosis and acidosis in groups B and C were produced during the phase of rapidly subsiding neuromuscular block, any enhancement of the block was considered significant but a decrease of the block only if it was followed by a spontaenous increase.

Table II. Blood values in metabolic alkalosis and acidosis

Rabbit no.	Tubocurarine dose mg/kg	Experimental condition	pH	pCO_2 mm Hg	CO_2 content meq/l	K meq/l
47	0.16	Initial value	7.33	34.2	18.9	
		After 7 meq Na_2CO_3	7.50	31.8	25.2	2.2
		10 min. after injection	7.38	35.8	22.0	
		After 7 meq HCl	7.12	40.0	14.0	3.2
		10 min. after injection	7.22	37.3	16.2	
48	0.18	Initial value	7.20			
		After 9 meq Na_2CO_3	7.62			3.2
		8 min. after injection	7.42			
		After 9 meq HCl	7.19			3.7
		10 min. after injection	7.30			
59	0.16	Initial value	7.44	34	23	2.8
		After 16 meq Na_2CO_3	7.90		58	5.7
		6 min. after injection	7.70			
		After 8 meq HCl	7.01			
60	0.16	Initial value	7.61	29	30	3.1
		After 16 meq Na_2CO_3	8.00		60	5.1
		4 min. after injection	7.59			
		After 7 meq HCl	6.90	50	9	5.0
63	0.17	Initial value	7.45	35	25	2.8
		After 6 meq Na_2CO_3	7.85	26	46	2.7
		5 min. after injection	7.68			
		After 9 meq HCl	6.96	44	10	
64	0.17	Initial value	7.49	33	26	2.5
		After 8 meq Na_2CO_3	7.76	28	42	2.7
		5 min. after injection	7.61			
		After 8 meq HCl	7.05	47	14	4.7
		6 min. after injection	7.37			
		After another 5.5 meq HCl	6.92	43	9	
65	0.17	Initial value	7.51	28	23	2.9
		After 8 meq Na_2CO_3	7.74	26	35	3.0
		5 min. after injection	7.67			
		After 8 meq HCl	7.18	28	11	2.8
		4 min. after injection	7.34			
		After another 7 meq HCl	6.98			

Results

Group I A. Fig. 1 illustrates the curve for spontaneous recovery of neuromuscular transmission after a single injection of tubocurarine in 1 exp. The pattern in the other 4 was similar with the maximal block developing within 5 min after the injection of tubocurarine. Thereafter the block steadily diminished

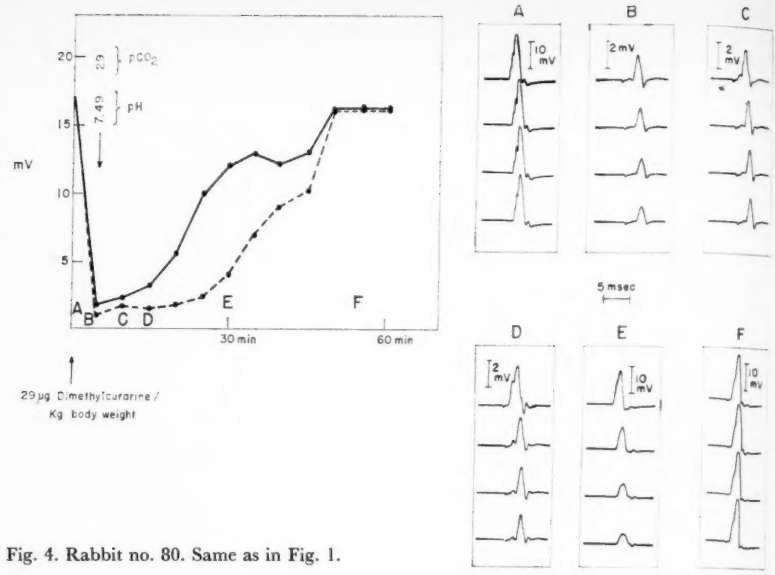

Fig. 4. Rabbit no. 80. Same as in Fig. 1.

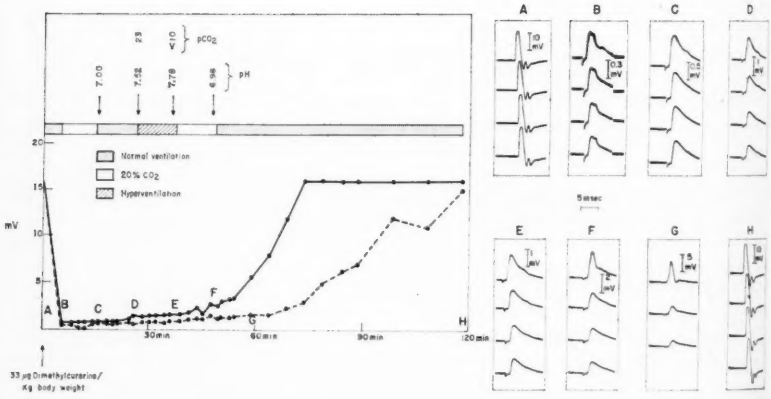

Fig. 5. Rabbit no. 85. Same as in Fig. 2.

Table III. Blood values in respiratory alkalosis and acidosis

Rabbit no.	Dimethyl tubocurarine µg/kg	Experimental condition	pH	pCO_2 mm Hg	CO_2 content meq/l
78	37	Normal ventilation	7.60	25	24
		Hyperventilation	7.76	26	37
		Normal ventilation	7.43	32	22
		5 % CO_2	7.17	>80	33
		10 % CO_2	7.10	>80	34
		20 % CO_2	6.96	>80	36
79	30	Normal ventilation	7.53	23.2	20
		20 % CO_2	6.95		
		10 % CO_2	7.10	80.5	26.9
		5 % CO_2	7.22	60.5	26.0
		Normal ventilation	7.49	26.6	20.5
		Hyperventilation	7.79		
84	30	Hyperventilation	7.72	12.4	15.7
		Normal ventilation	7.44	25.7	20.0
		5 % CO_2	7.23	50	22.9
		10 % CO_2	7.14		
		20 % CO_2	6.98		
85	33	20 % CO_2	7.00	>80	23
		Normal ventilation	7.52	23	19
		Hyperventilation	7.78	<10	12
		20 % CO_2	6.98		
105	12	Hyperventilation	7.66	11.8	12.9
		Normal ventilation	7.48	22	16.7
		20 % CO_2	6.92	>120	28.6
106	12	Hyperventilation	7.71		
		Normal ventilation	7.52	18.5	15.2
		20 % CO_2	7.02		

and had disappeared completely 30 to 70 min after the injection. These findings are in agreement with the results of other examiners (KALOW 1959). It is particularly important to stress that a spontaneous increase in the block was never observed during the recovery period.

Group I B. In this group 3 rabbits were excluded because of severe anoxia and subsequent metabolic acidosis; 6 exp. thus remain for analysis. In all of them the effect of tubocurarine dimished during hyperventilation. However, this occurred during the period of spontaneously subsiding block. The effect of hyperventilation was therefore not considered significant unless an increase in the block was observed during the period of normal ventilation following hyperventilation. This type of curve was found in 4 exp., while in 2 (rabbit

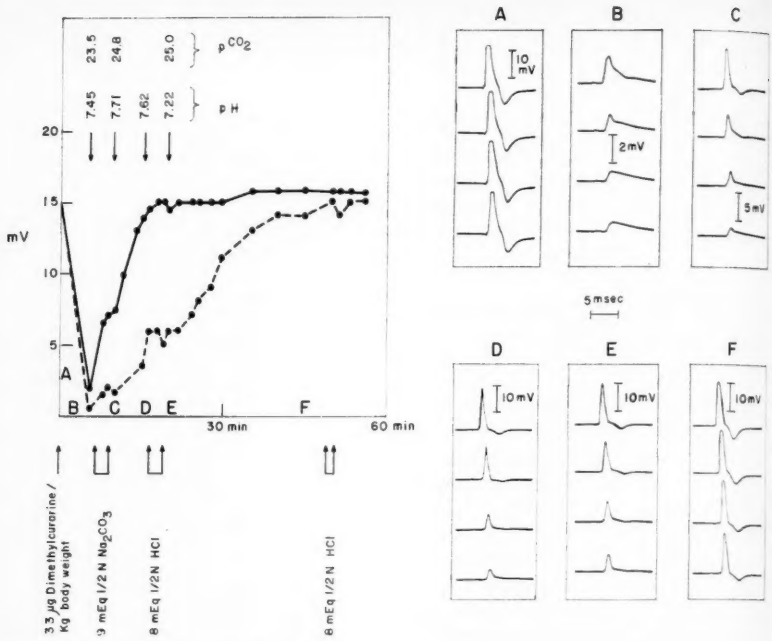

Fig. 6. Rabbit no. 77. Same as in Fig. 3.

no. 26, rabbit no. 30) the block continued to decrease during normal ventilation.

Administration of 5 % carbon dioxide did not influence the block significantly. An increase of the block was noted after either 10 or 20 % carbon dioxide in all 6 exp. and in 4 of 6 after both (rabbit no. 41 showed no change after 10 %, rabbit no. 30 not after 20 % carbon dioxide). These changes are significant as they occurred during a period when rapidly decreasing block could be expected. In 3 exp. Tensilon (0.02 to 0.03 mg) reversed the block during continued administration of carbon dioxide, while in another normal ventilation had the same effect. The result of a typical experiment is given in Fig. 2. Table I shows that respiratory alkalosis and acidosis were produced without significant change in the concentration of potassium in the plasma.

Group I C. The results were identical in all 7 rabbits in which metabolic alkalosis and acidosis were induced during curarization. The block decreased steadily during the injection of sodium carbonate and had almost disappeared 1 min after the end of the injection at which time the pH of the blood had increased. When the pH dropped, the block again increased. A marked en-

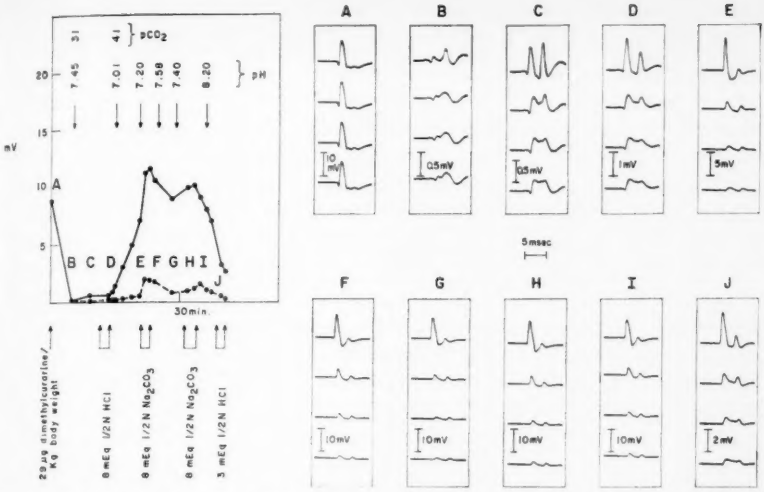

Fig. 7. Rabbit no. 76. Same as in Fig. 3.
Lines on illustrations have been darkenedf or photographic purpose.

hancement of the block occurred when the pH of the blood was lowered by i. v. injection of hydrochloric acid. When the pH rose spontaneously, the block again diminished. In 4 exp. a second injection of hydrochloric acid about 10 min later enhanced the block once more. In 2 exp. 0.1 mg of Tensilon given immediately after hydrochloric acid reversed the block, in another experiment sodium carbonate had the same effect.

The result of a typical experiment is given in Fig. 3. Table II summarizes the results of analysis of the blood samples showing that a marked change in pH was produced with only a small shift in carbon dioxide tension and no significant change in the concentration of potassium in the plasma.

Group II A. Fig. 4 illustrates the curve for spontaneous recovery of neuromuscular transmission after a single dose of dimethyl tubocurarine. The pattern in the other 3 exp. in this group was similar with the maximal block developing within 5 min. Thereafter the block steadily diminished and had disappeared completely 50 to 90 min later. The slight spontaneous increase once during the period after the first 5 min, seen in Fig. 4, was not observed in the other experiments. The blocking action of dimethyl tubocurarine was found to be stronger than that of tubocurarine. Its effect was also less uniform and the block varied greatly in severity and duration in different rabbits.

Group II B. The relative order between alkalosis and acidosis did not affect the results. No influence of respiratory alkalosis or acidosis on the block was

Table IV. Blood values in metabolic alkalosis and acidosis

Rabbit no.	Dimethyl tubocurarine μg/kg	Experimental condition	pH	pCO_2 mm Hg	CO_2 content meq/l
76	29	Initial value	7.45	31	22
		After 8 meq HCl	7.01	41	14
		7 min. after injection	7.20		
		After 8 meq Na_2CO_3	7.58		
		5 min. after injection	7.40		
		After 8 meq Na_2CO_3	8.20		
77	33	Initial value	7.45	23.5	16.4
		After 9 meq Na_2CO_3	7.71	24.8	30.7
		7 min. after injection	7.62		
		After 8 meq HCl	7.22	25	10.9
81	33	Initial value	7.49	26.6	20.5
		After 8 meq HCl	6.88	41.0	8.7
		7 min. after injection	7.24		
		After 8 meq Na_2CO_3	7.58	27	25.9
		7 min. after injection	7.42		
82	29	Initial value	7.49	31	24
		After 8 meq Na_2CO_3	7.75	24	33
		6 min. after injection	7.61		
		After 7.5 meq HCl	7.12	38	14
101	20	Initial value	7.32	48	25.8
		After 8 meq Na_2CO_3	7.80	30	46.7
		5 min. after injection	7.51		
		After 8 meq HCl	7.20	48	19.9
102	15	Initial value	7.60	19	19
		After 9 meq Na_2CO_3	7.95	20	44
		6 min. after injection	7.75		
		After 5.5 meq HCl	6.88	34	7
103	23	Initial value	7.45	29	20
		After 9 meq Na_2CO_3	7.78	27	40
		6 min. after injection	7.60		
107	12	Initial value	7.52	22	18
		After 7.5 meq Na_2CO_3	7.75	24	33
		8 min. after injection	7.69		
		After 9 meq HCl	6.80	44	8
		10 min. after injection	7.20		
108	36	Initial value	7.43	26	18
		After 9 meq HCl	6.88	41	9
		8 min. after injection	7.18		
		23 min. after injection	7.28		
	44	Another dose of dimethyl tubocurarine			
		After 7 meq HCl	6.89		
109	23	Initial value	7.58	18.7	17.5
		After 4.5 meq HCl	7.21	15.5	6.6
		7 min. after injection	7.22		

noted in 5 of 6 rabbits. In 2 rabbits 20 % carbon dioxide was administered twice without effect. In a single experiment (rabbit no. 84) administration of 20 % carbon dioxide was accompanied by an increase in the effect of dimethyl tubocurarine; in none was it influenced by hyperventilation.

Fig. 5 illustrates the result in one of the negative experiments. That pure or almost pure respiratory alkalosis and acidosis had been produced was confirmed by analysis of the blood samples. The results are listed in Table III.

Group II C. The relative order between alkalosis and acidosis did not affect the results. No influence on the block of metabolic alkalosis or acidosis was noted in 6 of 10 rabbits. The block diminished in 3 rabbits (no. 76, 101, 103) during alkalosis to increase again when the pH of the arterial blood dropped spontaneously. This change, which could be produced twice in 1 rabbit (no. 76), was weak and approached the marked effect on a block produced by tubocurarine in a single experiment (rabbit no. 103). A slight enhancement of the block was noted during metabolic acidosis in 3 rabbits (no. 76, 103, 107), in none approaching the marked effect on a block produced by tubocurarine.

Fig. 6 illustrates the result in a negative experiment and Fig. 7 in an experiment showing a weak influence by metabolic alkalosis and acidosis. Table IV summarizes the results of the analysis of the blood samples showing that a marked change in pH was produced with only a small shift in carbon dioxide tension.

Discussion

I. The results of our experiments with metabolic alkalosis and acidosis indicate that the blocking action of tubocurarine varies with changes in the pH of the blood, increasing at a low pH and decreasing at a high pH. The shift in the block induced by respiratory alkalosis and acidosis was of the same type but of less magnitude. This can be explained by a smaller (only during alkalosis, see Table I and II) and a slower (both during alkalosis and acidosis) change in blood pH; in metabolic alkalosis and acidosis a shift in the pH of the blood was induced over 2—3 min, whereas it required 10 min to occur during respiratory alkalosis and acidosis. The time factor is important as the changes in pH were produced during the phase of rapidly decreasing block after a single injection of tubocurarine. Furthermore, the respiratory experiments were extended over a longer period, as 2 or 3 different concentrations of carbon dioxide were tested. The reaction to a shift in the pH of the blood is thus qualitatively the same whether or not this shift is induced by changes in carbon dioxide tension.

The effect of changes in carbon dioxide and blood pH (Gamstorp and Vinnars, 1961) on the neuromuscular transmission have previously been shown to be minimal and cannot account for the altered sensitivity to tubocurarine. Release of epinephrine, known to influence the effect of curare

(NAESS and SIRNES 1953), could not explain the decrease in block during alkalosis. Furthermore, the increased curare block during acidosis was reversible either by administration of Tensilon or by raising the pH of the blood. It can thus be concluded that a shift in the pH of the blood influences the effect of tubocurarine principally by changing the apparent potency of the drug.

The results of PAYNE's (1958) experiments on the sensitivity to tubocurarine during respiratory acidosis in cats are similar to ours. Our findings during respiratory alkalosis correspond to the clinical observation by DUNDEE (1952) that hyperventilation increases the dose of tubocurarine required for abdominal surgery during the first hour of the operation.

Our results during metabolic alkalosis and acidosis are, however, contrary to PAYNE's (1960). One explanation might be that different species were used, but non-depolarizing blocking agents are known to act in essentially the same way in both these species (FOLDES 1959). The fact that PAYNE recorded the mechanical and we the electrical response might play a role, as metabolic changes could conceivably influence the contractility of the muscle which would not be reflected in the electrical response. This could scarcely be the whole explanation since PAYNE (1960) found a variable influence of metabolic alkalosis and acidosis on the mechanical response in the uncurarized animal. Of greater importance may be that by using stronger solutions and faster injection rates we produced a more rapid shift in the pH of the blood. This allowed us to measure changes in the effect of tubocurarine during the phase of rapidly diminishing block after a single injection of the drug. Finally, PAYNE does not state whether he used a supramaximal stimulus and maintained is so throughout the experiment. He produced metabolic alkalosis by infusing sodium bicarbonate thereby also raising the carbon dioxide tension which will increase the stimulus necessary to evoke a maximal response (GAMSTORP and VINNARS 1961). If the applied stimulus was not above the raised level necessary for evoking a maximal response, this mechanism might be responsible for an apparent increase in the block.

II. As dimethyl tubocurarine is manufactured from tubocurarine, a variable trace of the latter drug may conceivably remain in the former, thereby explaining its variable potency. In most of the experiments changes in blood pH did not influence the block occurring after injection of dimethyl tubocurarine; any influence found was always in the same direction but much weaker than on the block produced by tubocurarine. A small, variable amount of tubocurarine in dimethyltubocurarine could explain these findings thus making it unnecessary to assume any influence by changes in blood pH on the block produced by dimethyl tubocurarine. Changes in the partial pressure of carbon dioxide in arterial blood did not influence the block.

Our results are in disagreement with those obtained by PAYNE (1959, 1960) who found the block produced by dimethyl tubocurarine to increase during alkalosis and decrease during acidosis. We are unable to explain this discrepancy.

Tubocurarine and dimethyl tubocurarine are both non-depolarizing blocking agents with principally the same effect at the neuromuscular junction. Yet only the block produced by tubocurarine is influenced by changes in blood pH. The difference is well explained by KALOW's (1954) observation that the ionization of tubocurarine but not of dimethyl tubocurarine changes with the pH of the solution.

We are indebted to Dr. PHILIP R. DODGE and Dr. ROBERT S. SCHWAB, Neurology Service, and to Dr. HENRY K. BEECHER, Dr. JOHN P. BUNKER and Dr. HENRIK H. BENDIXEN, Anaesthesia Service, and Dr. JOHN D. CRAWFORD, Children's Medical Service for help and encouragement throughout the work and for placing equipment and laboratory facilities at our disposal.

References

DUNDEE, J. W., Influence of controlled respiration on dosage of thiopentone and d-tubocurarine chloride required for abdominal surgery. *Brit. med. J.* 1952. *2.* 893—896.

FOLDES, F. F., Factors which alter the effects of muscle relaxants. *Anesthesiology* 1959. *20.* 464—504.

GAMSTORP, I. and E. VINNARS, Studies in neuromuscular transmission. I. Influence on neuromuscular transmission of alkalosis and acidosis. *Acta physiol. scand.* 1961. *53.* 142—150.

GRAY, T. C. and E. S. N. FENTON, Carbon dioxide retention simulating curarization. *Brit. med. J.* 1954. *1.* 820—820.

JOHANSEN, S. H. and P. F. OSGOOD, Influence of hypercarbia on the activity of neuromuscular blocking agents in the cat. Personal communication 1960.

KALOW, W., Influence of pH on the ionization and biological activity of d-tubocurarine. *J. Pharmacol.* 1954. *110.* 433—442.

KALOW, W., Distribution, destruction and elimination of muscle relaxants. *Anesthesiology.* 1959. *20.* 505—518.

NAESS, K. and T. SIRNES, Synergistic effect of adrenaline and d-tubocurarine on neuromuscular transmission. *Acta physiol. scand.* 1953. *29.* 293—306.

PAYNE, J. P., The influence of carbon dioxide on the neuromuscular blocking activity of relaxant drugs in the cat. *Brit. J. Anaesth.* 1958. *30.* 206—216.

PAYNE, J. P., Changes in neuromuscular blocking activity of tubocurarine and dimethyl tubocurarine induced by the administration of carbon dioxide. *Acta anaesth. scand.* 1959. *3.* 53—58.

PAYNE, J. P., The influence of changes in blood pH on the neuromuscular blocking properties of tubocurarine and dimethyl tubocurarine in the cat. *Acta anaesth. scand.* 1960. *4.* 83—90.

SCURR, C. F., Carbon dioxide retention simulating curarization. *Brit. med. J.* 1954. *1.* 565—566.

Acta physiol. scand. 1961. 53. 174—184

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Restoration of Vasoconstrictor Effects in Reserpinized Cats[1]

By

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Abstract

ROSELL, S. and G. SEDVALL, *Restoration of vasoconstrictor effects in reserpinized cats*. Acta physiol. scand. 1961. *53*. 174—184. — Reserpine completely abolishes the effects of vasoconstrictor nerve stimulation (BURN and RAND 1958, ROSELL and ROSÉN 1961). Dopa, dopamine, noradrenaline and adrenaline infused intra-arterially 20—24 hours after the administration of reserpine partially restored the responses to stimulation of the sympathetic chain. The responsiveness persisted for at least one hour after termination of dopa, dopamine and noradrenaline infusion. With adrenaline, however, vasoconstrictor responses to sympathetic chain stimulation persisted for only a short period after termination of the infusion. The threshold dose for dopa was 2 mg, for dopamine and noradrenaline 25 μg, and for adrenaline 100 μg.

The data are considered to support the hypothesis of BURN and RAND (1960) that the adrenergic nerve terminals or adjacent tissue in reserpinized animals have the ability to store infused dopamine and noradrenaline. Adrenaline seems to be stored to a much lesser extent. Dopamine is presumably converted to noradrenaline, which is then released when the sympathetic chain is stimulated. The weak restoration of vasoconstrictor responses following infusion of dopa is of interest. The degree of restoration of the vasoconstrictor effects following infusion of noradrenaline is presumably related to the time elapsed between the administration of reserpine and the infusion.

[1] A preliminary report of this work was presented at the Second Scandinavian Summer Meeting of Biochemistry, Medical Chemistry, Pharmacology and Physiology. Joint Meeting with The Biochemical Society. Turku, in August, 1959.

Reserpine blocks the effects of sympathetic vasoconstrictor nerve activity, apparently due to a depletion of the adrenergic transmitter substance at the nerve terminals (BERTLER, CARLSSON and ROSENGREN 1956, MUSCHOLL and VOGT 1958, BURN and RAND 1958). On the other hand, the cholinergic vasodilator nerves appear to be functionally intact in reserpinized animals. After reserpine treatment, therefore, stimulation of the sympathetic chain produces vasodilatation in the skeletal muscle vessels (ROSELL and ROSÉN 1961).

In the circulatory studies on reserpinized cats reported by ROSELL and ROSÉN (1961) the low blood pressure was a disturbing factor. In order to raise the blood pressure to a more adequate level intravenous infusion of noradrenaline was tried. This unexpectedly led to reappearance of signs of vasoconstrictor activity. In the hope of accounting for this restoration of vasoconstrictor effects a series of experiments was instituted, and it was subsequently disclosed that the vasoconstrictor effects could be partially restored by intra-arterial infusion of adrenaline, noradrenaline, dopamine and dopa. While the work was in progress BURN and RAND (1958, 1960) reported that infusion of noradrenaline restored the vasoconstrictor effects in the perfused hind leg of the reserpinized dog. They noted, furthermore, that the responses to stimulation of fibers to the nictitating membrane and to the iris were restored by infusion of dopamine or dopa.

The present study deals exclusively with the effects of dopa, dopamine, noradrenaline and adrenaline on the vasoconstrictor responses to sympathetic chain stimulation in skeletal muscle vessels of the reserpinized cat.

Methods

The experiments were performed on cats weighing 2.0 to 4.4 kg under urethane (600—1,200 mg/kg) anesthesia. The trachea was cannulated. The arterial pressure was recorded from one of the carotids by a mercury manometer or a Statham pressure transducer (P 23 AA). Reserpine (5 mg/kg) was administered subcutaneously 20—24 hours before the experiments.

Blood flow was measured by cannulating the femoral artery and directing the blood through a silicone filled drop chamber operating an ordinate writer (LINDGREN 1958). The blood re-entered the hind leg via the cannulated distal stump of the same artery. In order to measure only muscle blood flow the hind leg was skinned, and a tight ligature around the ankle isolated the paw from the circulation. In most experiments blood flow was recorded from each skinned hind limb separately. In one limb intra-arterial infusions were made through a side arm of the arterial drop chamber. The other limb served as a control. To prevent clotting, heparin (25 mg/kg) was given i. v. Dextran was administered i. v. as required to compensate for blood loss.

In those experiments in which blood flow was measured in both hind limbs, both sympathetic chains were isolated via the anterior approach and subsequently cut. When only one hind limb was prepared for blood flow measurements, the ipsilateral sympathetic chain was isolated and cut. A bipolar silver electrode was applied to the distal part of the transected sympathetic chain at the level of L_4—L_5. When both chains were stimulated, they were placed on the same electrode. Supramaximal stimulation voltage produced by a Grass Model S4 stimulator was used throughout.

Rectal temperature was maintained at 36—37° C by means of a heating lamp.

Usually the measurements were recorded on a kymograph. In some experiments a Grass Polygraph was used as a recording instrument.

The vascular resistance was calculated as the ratio of arterial pressure to flow.

Substances used:

Reserpine (Serpedin, Pharmacia) 2.5 %.
Dopa (L (—) Dihydroxy-phenylalanin, Hoffmann-La Roche).
Dopamine (3-Hydroxy-tyramin-hydrochlorid, Hoffmann-La Roche).
Adrenaline (L-adrenaline, ACO).
Noradrenaline (Noradrenalin-Conc., Byk-Gulden, L-noradrenalin. bitartr.).
Dextran (Macrodex, Pharmacia).
Heparin (Heparin, Vitrum) 5 %.
Atropine (Atropini sulfas, Sw. Ph. XI).

The drugs for intra-arterial infusions were dissolved in 0.9 per cent saline. The following concentrations were used:

Dopa 1 mg/ml; Dopamine, Noradrenaline and Adrenaline 10 μg/ml.

The catechol amines are expressed as bases in data concerning dosage given.

Results

1. *Intra-arterial Infusion of Noradrenaline*

In 18 reserpinized cats noradrenaline was infused i. a. The total dose varied between 15—100 μg and the infusion rate between 1—2 μg/min. After infusion of a minimum of 25 μg noradrenaline, electrical stimulation of the sympathetic chain induced vasoconstrictor responses. Consistent results were obtained in all experiments. Fig. 1 illustrates a typical experiment. Numbers 1, 2 and 3 show the vasodilator responses in a reserpinized cat at different stimulation frequencies. The effects could be blocked by atropine given i. v. (4, 5, 6). After atropine, stimulation elicited no consistent change in blood flow, neither vasodilatation nor vasoconstriction was apparent, presumably because reserpine had completely abolished the vasoconstrictor responses and atropine the vasodilator responses (ROSELL and ROSÉN 1961). Between 6 and 7 50 μg of noradrenaline was infused into the left femoral artery. It will be seen (7, 8 and 9) that 10 min after completion of the infusion, stimulation of the sympathetic chain evoked pronounced vasoconstrictor effects in the left hind limb. Numbers 10, 11 and 12 indicate the vasoconstrictor responses 60 min after termination of the noradrenaline infusion. Vasoconstriction again occurred in the left hind limb, although the effects this time were less pronounced. After 120 min, however, the blood flow showed no more than a tendency to decrease on sympathetic chain stimulation. Between numbers 15 and 16 noradrenaline (25 μg) was again infused into the left femoral artery. The series of stimuli of the sympathetic chain (16, 17, 18), initiated 10 min after termination of the infusion, show that it was once more possible to elicit vasoconstrictor effects.

In Fig. 2 the stimulation frequency-response curves following infusion of 100 μg of noradrenaline in reserpinized cats are compared with equivalent

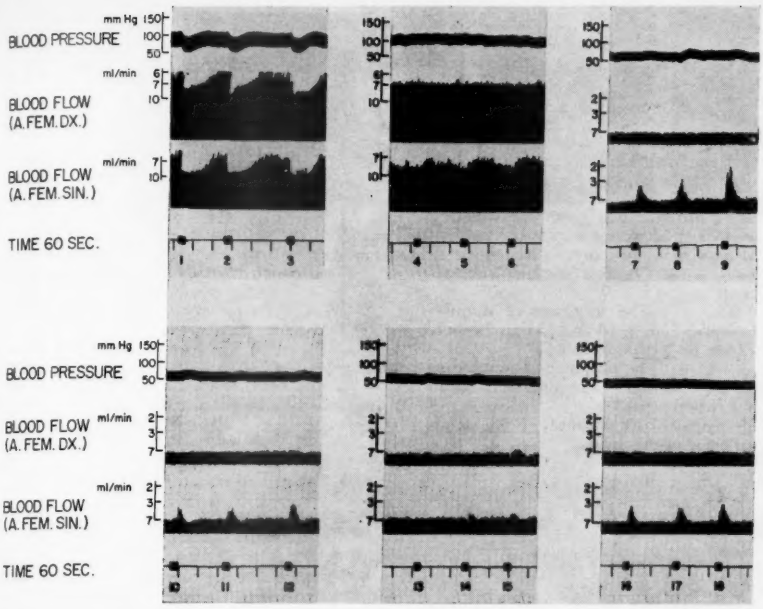

Fig. 1. Cat 3.0 kg. Reserpine 5 mg/kg. Urethane 600 mg/kg. Vasomotor responses in skeletal muscle to stimulation of both sympathetic chains at L_4—L_5 before and after infusion of noradrenaline into the left femoral artery.
1. Stimulation, 2.5 V, 8 imp/sec 15 sec.
2. » 2.5 V, 10 imp/sec 15 sec.
3. » 2.5 V, 20 imp/sec 15 sec.
Between 3 and 4 0.5 mg/kg atropine intravenously.
4. = 1, 5. = 2, 6. = 3.
Between 6 and 7, 50 μg noradrenaline infused for 50 min.
7. = 1. 10 min after infusion had ceased.
8. = 2, 9. = 3.
10. = 1. 60 min after infusion had ceased.
11. = 2, 12. = 3.
13. = 1. 120 min after infusion had ceased.
14. = 2, 15. = 3.
Between 15 and 16, 25 μg noradrenaline infused for 25 min.
16. = 1. 10 min after infusion had ceased.
17. =2, 18. = 3.
Note the vasoconstrictor responses after infusion of noradrenaline.

curves from "normal" cats. Two factors influenced the observed stimulation responses: (1) the elapsed time between the end of the infusion and the stimulation and (2) preceding stimulation. Each stimulation appeared to reduce the available amount of transmitter substance to such a degree that the response to the next stimulation was less than predicted. In order to gauge the effects of these two factors on the stimulation responses, two different sequences of

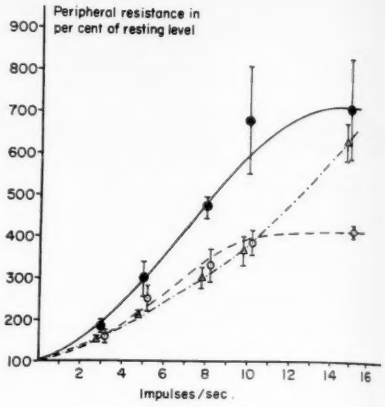

Fig. 2. *Peripheral resistance in percent of resting level during stimulation of the sympathetic chain after intra-arterial infusion of 100 μg noradrenaline in reserpinized cats.*
The series of stimuli started 10 min after the end of infusion. Two minutes between consecutive stimuli. Administration of 5 mg/kg reserpine subcutaneously 20—24 hours before the experiment. Both in control animals (not reserpinized, no infusion) and in reserpinized animals 0.5 mg/kg atropine was given intravenously in order to block the action of vasodilator nerves.
●———● Controls (mean of 4 experiments).
○– – – –○ The sequence of stimulations was 3, 5, 8, 10 and 15 impulses/sec. (Mean of 4 experiments.)
△— · —△ The sequence of stimuli was 15, 10, 8, 5 and 3 impulses/sec. (Mean of 4 experiments.)
The vertical bars represent ± the standard error of the mean.

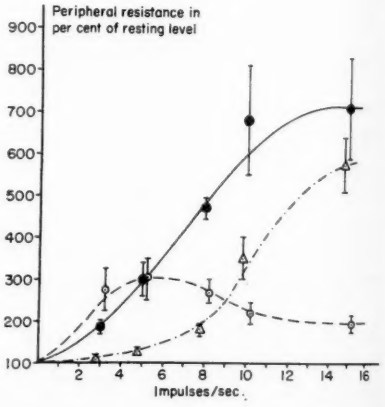

Fig. 3. *Peripheral resistance in percent of resting level during stimulation of the sympathetic chain after intra-arterial infusion of 100μg adrenaline in reserpinized cats.*
●———● Controls. (Mean of 4 experiments.)
○– – – –○ The sequence of stimuli was 3, 5, 8, 10 and 15 impulses/sec. (Mean of 4 experiments.)
△— · —△ The sequence of stimuli was 15, 10, 8, 5 and 3 impulses/sec. (Mean of 4 experiments.)
For further details see legend to fig. 2.

stimuli were given in reserpinized animals. One frequency-response curve shows the changes in peripheral resistance when the series of stimuli opened with the lowest frequency (3 imp/sec), followed by higher ones (5, 8, 10 and 15 imp/sec). The other curve shows changes in peripheral resistance when the sequence of stimuli was reversed (15, 10, 8, 5 and 3/sec respectively). Although both curves differ from the normal one they do not, except in one instance, differ significantly from each other. The one exception is that at the highest frequency (15/sec) the percentual change in peripheral resistance was greater when the series began with stimuli of that frequency than when the sequence was reversed.

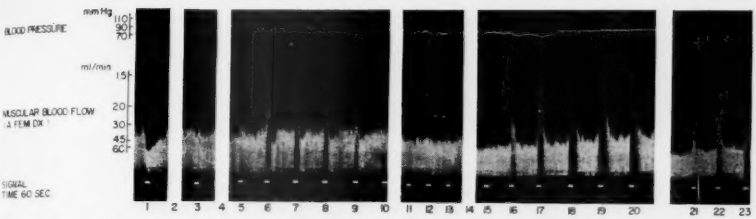

Fig. 4. Cat 2.5 kg. Reserpine 5 mg/kg. Urethane 600 mg/kg. Vasomotor responses in skeletal muscle to stimulation of right sympathetic chain (L —L_5) before and after infusion of adrenaline and noradrenaline into the right femoral artery.
1. Stimulation, 4 V, 15 imp/sec.
2. 0.5 mg/kg atropine intravenously.
3. = 1.
4. 100 μg adrenaline infused for 80 min.
5. Stimulation, 4 V, 1 imp/sec. 10 min after infusion had ceased.
6. Stimulation, 4 V, 3 imp/sec.
7. » 4 V, 5 imp/sec.
8. » 4 V, 8 imp/sec.
9. » 4 V, 10 imp/sec.
10. » 4 V, 15 imp/sec.
11. = 6. 30 min after infusion had ceased.
12. = 7, 13. = 8.
14. 100 μg noradrenaline infused for 85 min.
15. = 5. 10 min after infusion had ceased.
16. = 6, 17. = 7, 18. = 8, 19. = 9, 20. = 10.
21. = 6. 30 min after infusion had ceased.
22. = 7, 23. = 8.
Note the rapid disappearance of vasomotor responses after adrenaline infusion, compared with the magnitude of the responses after noradrenaline infusion.

2. *Intra-arterial Infusion of Adrenaline*

Adrenaline (45—150 μg) was infused into 13 reserpinized cats. The infusion rate varied between 1.0—1.7 μg/min. Following a dose of 100 μg or more, sympathetic chain stimulation induced vasoconstrictor responses. Fig. 3 shows the frequency-response curves after infusion of 100 μg of adrenaline. It is apparent that the frequency-response curves differ from the curves of "normal" cats, both in the stimulation series opening with a low and that opening with a high frequency. For series opening with a low frequency the change in peripheral resistance was less at 10 and 15 imp./sec than at 3/sec or 5/sec. This phenomenon is illustrated in Fig. 4. In this particular experiment both adrenaline and noradrenaline were infused i. a. Ten minutes after the infusion of adrenaline a series of sympathetic chain stimuli was initiated. The vasoconstrictor responses were greatest at 3 and 5 imp./sec after which they progressively diminished until, at 15/sec, they had almost disappeared. A series of stimuli initiated 30 min after the end of the infusion produced no change in blood flow (11, 12 and 13). An identical dose of noradrenaline (100 μg) was then infused. Following noradrenaline there was no decrease of the vasoconstrictor responses at higher stimulation frequencies. Furthermore, vasoconstrictor responses still

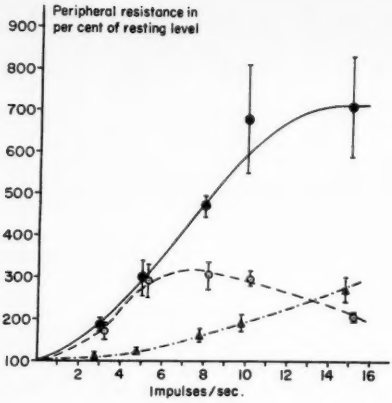

Fig. 5. *Peripheral resistance in percent of resting level during stimulation of the sympathetic chain after intra-arterial infusion of 100 μg dopamine.*
●———● Controls. (Mean of 4 experiments.)
○– – – –○ The sequence of stimuli was 3, 5, 8, 10 and 15 impulses/sec. (Mean of 4 experiments.)
△– · –△ The sequence of stimuli was 15, 10, 8, 5 and 3 impulses/sec. (Mean of 4 experiments.)
For further details see legend to fig. 2.

persisted 30 min after the infusion of noradrenaline had terminated. It would seem therefore, that infused adrenaline may be stored to a much lesser extent than infused noradrenaline at the nerve endings of the vasoconstrictor nerves in reserpinized cats.

3. *Intra-arterial Infusion of Dopamine*

In 18 reserpinized cats dopamine was administered i. a., the doses varying between 25—4,000 μg and the infusion rates between 2.5—6.7 μg/min. After low doses (25—400 μg) the vasoconstrictor responses to sympathetic chain stimulation were restored. With doses of about 400 μg or more, on the other hand, no vasoconstrictor responses were manifest at the lower stimulation frequencies (1, 3 and 5/sec). With still higher doses (2.5—4 mg) no vasoconstrictor responses whatsoever could be elicited in the infused hind limb. Studies on non-reserpinized cats showed that dopamine in comparatively high doses (1—4 mg i. a.) had a sympatholytic action of at least one hour's duration, apparently as a result of competition for the receptor sites between the infused material, dopamine and the released transmitter substance. The failure of dopamine in high doses to restore the vasoconstrictor effects in the infused leg of a reserpinized cat may well be attributable to this sympatholytic action. Fig. 5 shows the stimulation frequency-response curves after infusion of 100 μg dopamine. It will be seen that even dopamine may to some extent restore the effects of the vasoconstrictor nerve activity in reserpinized cats. It was impossible, however, to elicit vasoconstrictor responses of the same nature as those obtained in "normal" cats.

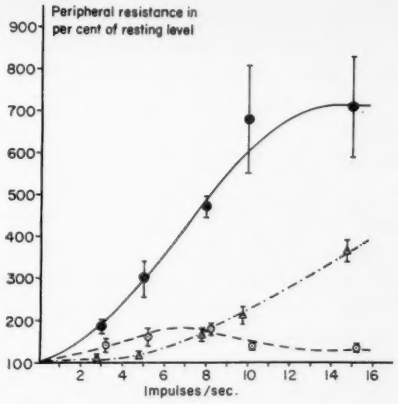

Fig. 6. *Peripheral resistance in percent of resting level during stimulation of the sympathetic chain after intra-arterial infusion of 5 mg dopa.*
●——● Controls. (Mean of 4 experiments.)
○– – – –○ The sequence of stimuli was 3, 5, 8, 10 and 15 impulses/sec. Mean of 4 experiments.)
△– · – △ The sequence of stimuli was 15, 10, 8, 5 and 3 impulses/sec. (Mean of 4 experiments.)
For further details see legend to fig. 2.

4. *Intra-arterial Infusion of Dopa*

The vasoconstrictor effects in reserpinized cats were also reactivated by infusion of dopa, though only after high doses (2 mg or more. Infusion rate: 0.08—0.25 mg/min). In 11 experiments dopa was infused in doses varying between 0.075—5.8 mg. Fig. 6 shows the stimulation frequency-response curves following infusion of 5 mg i. a. By comparison with the vasoconstrictor response obtained on stimulation of the sympathetic chain in non-reserpinized cats, the effects at all frequencies were slight.

Discussion

The biosynthesis of noradrenaline and adrenaline seems to proceed along the pathway suggested by BLASCHKO (1939). By introduction of a phenolic OH group into tyrosine, dopa is formed and in turn is decarboxylated to dopamine. Dopamine is then hydroxylated to noradrenaline and this in turn is methylated to adrenaline. We have studied the effects of dopa, dopamine, noradrenaline and adrenaline on the responses to stimulation of the vasoconstrictor nerves of skeletal muscle vessels in reserpinized cats. Each of the substances tested is in fact able to restore vasoconstrictor effects. There are, however, important quantitative differences. From Fig. 1 it is evident that vasoconstrictor effects could be elicited at least one hour after the infusion of noradrenaline had terminated. The same was true of dopa and dopamine but not of adrenaline. These results are consistent with those of AXELROD (1960 a). He concluded that a greater fraction of administered noradrenaline than ad-

ministered adrenaline appears to be bound to the tissues. Furthermore, EULER (1956, 1958) and SCHÜMANN (1958) on the basis of results obtained with homogenates of sympathetic nerves (bovine splenic nerves), showed that these nerves contain noradrenaline but not adrenaline granules. Moreover, FOLKOW and UVNÄS (1948) obtained evidence that the transmitter substance at the vasoconstrictor nerve endings consisted of noradrenaline rather than adrenaline.

It is interesting to note that sympathetic stimulation following administration of adrenaline induced vasoconstriction, never vasodilatation. As is well known adrenaline in low concentrations elicits dilatation in the skeletal muscle vessels of the cat but in high concentrations induces vasoconstriction. It is not likely that adrenaline was demethylated to noradrenaline which was then released by stimulation, since there is little evidence for the formation of noradrenaline from adrenaline (BACQ 1960, AXELROD 1960 b). The interpretation may be that the release of adrenaline by stimulation was presumably confined to certain restricted areas with a specialized type of receptor site whereas upon infusion of adrenaline there is a general distribution of the drug.

Infusion of dopamine also reactivated the adrenergic vasoconstrictor responses. The pressor action of dopamine is 50 to 100 times less than that of noradrenaline (BALZER and HOLTZ 1956). On the other hand, the threshold doses for restoration of the effects of the vasoconstrictor nerve impulses were about the same for noradrenaline and dopamine (25 μg). The major difference in the pressor effects of the two substances despite the near parity of the threshold doses suggests the conversion of infused dopamine to a more potent pressor amine, probably noradrenaline, and the subsequent release of the latter on stimulation of the vasoconstrictor nerves.

Dopa likewise had the capacity to reactivate the vasoconstrictor nerves. It is highly unlikely that dopa *per se*, which exhibits no direct vasoactive properties when administered intravenously or intra-arterially, would restore the effects of vasoconstrictor nerve activity. It is reasonable to suppose, therefore, that the amino acid dopa underwent conversion to an amine. One possibility is that dopa was decarboxylated to dopamine and the latter, in turn, hydroxylated to noradrenaline. In view of the quantitative relationships it is almost impossible, however, to conclude with assurance which substance was released during vasoconstrictor nerve stimulation after infusion of dopa. Even after large doses of dopa (5 mg i. a.) the vasoconstrictor responses did not exceed those observed following infusion of 100 μg dopamine. Assuming that formation of dopa to noradrenaline occurred in the hind leg, this may imply that at most 2 per cent of the infused dopa was converted to dopamine. The explanation of this small conversion has not yet been sought. In the sequence of reactions from tyrosine to noradrenaline the fastest reaction is presumably the decarboxylation of dopa to dopamine (for references see HOLTZ 1959). Thus the small conversion is probably not due to a slow rate of decarboxylation of dopa to dop-

amine. Moreover HOLTZ and WESTERMANN (1956) found a high dopadecarboxylase activity in splenic nerves, indicating that sympathetic nerves may form dopamine from dopa. GOODALL and KIRSHNER (1958), using radioactive dopa and tyrosine, studied the formation of adrenaline and noradrenaline in minced sympathetic nerves and ganglia. They reported that dopamine and noradrenaline were formed. These studies of HOLTZ and WESTERMANN and of GOODALL and KIRSHNER took form of *in vitro* experiments on homogenized splenic nerves. To some extent our results seem to support these findings. Thus it is probable that in the cat adrenergic vasoconstrictor nerve terminals or adjacent tissue form noradrenaline from dopamine; whereas the evidence that the same tissue in reserpinized cats form dopamine from dopa is by no means conclusive.

BURN and RAND (1960) suggested that the restoration of the effects of sympathetic stimulation following an infusion of noradrenaline is due to the retention of noradrenaline in some form. Moreover they considered it likely that infused precursors undergo conversion in the direction of noradrenaline before they act. However, MUSCHOLL (1960) has shown that in reserpinized rats (5 mg/kg) infusions of noradrenaline did not produce any increase of the noradrenaline concentration in the heart. On the other hand, in normal rats, the heart concentration of noradrenaline increased considerably after infusion of noradrenaline. These results of MUSCHOLL are in agreement with the assumption that reserpine blocks the active transport of catechol amines to storage granules (HUGHES and BRODIE 1959, BERTLER, ROSENGREN and ROSENGREN 1960). At first glance our experimental results and those of BURN and RAND appear to contradict this hypothesis. However, experiments have been performed in which restoration of vasoconstrictor effects was tested 5 hours after the administration of reserpine. After such a short interval noradrenaline (50 μg i. a.) failed to restore the vasoconstrictor response of sympathetic chain stimulation (unpublished observations). Furthermore, in the experiments described in this paper 100 μg of noradrenaline could not restore the effect of vasoconstrictor nerve stimulation to a normal level (Fig. 2). It is thus conceivable that the action of reserpine had partially subsided during the long interval between the administration of reserpine and the experiment (20—24 hours). In the experiments of MUSCHOLL reserpine was also administered 20 hours before beginning the experiment. However, the duration of the action of reserpine might very well vary according to species and organ. Thus the experiments presented and those of BURN and RAND do not seem to contradict the hypothesis that reserpine blocks the active transport of catechol amines to storage granules.

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References

AXELROD, J., The fate of adrenaline and noradrenaline. In Adrenergic Mechanisms. Churchill Ltd. London, 1960 a. 28—39.

AXELROD, J., In Adrenergic Mechanisms. Churchill Ltd. London, 1960 b. p. 350.

BACQ, Z. M., In Adrenergic Mechanisms. Churchill Ltd. London, 1960 p. 350.

BALZER, H. and P. HOLTZ, Beeinflussung der Wirkung biogener Amine durch Hemmung der Aminoxydase. *Arch. exp. Path. Pharmak.* 1956. *227.* 547—558.

BERTLER, Å., A. CARLSSON and E. ROSENGREN, Release by reserpine of catechol amines from rabbit's hearts. *Naturwissenschaften* 1956. *43.* 521.

BERTLER, Å., A.-M. ROSENGREN and E. ROSENGREN, In vivo uptake of dopamine and 5-hydroxytryptamine by adrenal medullary granules. *Experientia* (Basel) 1960. *16.* 418.

BLASCHKO, H., The specific action of l-dopa decarboxylase. *J. Physiol.* (Lond.) 1939. *96.* 50 P.

BURN, J. H. and M. J. RAND, The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol.* (Lond.) 1958. *144.* 314—336.

BURN, J. H. and M. J. RAND, The effect of precursors of noradrenaline on the response to tyramine and sympathetic stimulation. *Brit. J. Pharmacol.* 1960. *15.* 47—55.

EULER, U. S. v., Noradrenaline and histamine in separate fractions of homogenized adrenergic nerves. *XXth Int. Physiol. Congr.* Brussels 1956. 933—934.

EULER, U. S. v., Distribution and metabolism of catechol hormones in tissues and axons. *Recent Progr. Hormone Res.* 1958. *14.* 483—507.

FOLKOW, B. and B. UVNÄS, The chemical transmission of vasoconstrictor impulses to the hind limbs and the splanchnic region of the cat. *Acta physiol. scand.* 1948. *15.* 365—387.

GOODALL, Mc C. and N. KIRSHNER, Biosynthesis of epinephrine and norepinephrine by sympathetic nerves and ganglia. *Circulation* (N. Y.) 1958. *17.* 366—371.

HOLTZ, P., Role of l-dopa decarboxylase in the biosynthesis of catecholamines in nervous tissue and the adrenal medulla. In Symposium on Catecholamines. The Williams and Wilkins Company. Baltimore, 1959. 317—329.

HOLTZ, P. and E. WESTERMANN, Über die Vorstufe des Nor-adrenalins im Nebennierenmark und Nervengewebe. *Biochem. Z.* 1956. *327.* 502—506.

HUGHES, F. B. and B. B. BRODIE, The mechanism of serotonin and catecholamine uptake by platelets. *J. Pharmacol.* 1959. *127.* 96—102.

LINDGREN, P., An improved method for drop recording of arterial or venous blood flow. *Acta physiol. scand.* 1958. *42.* 5—11.

MUSCHOLL, E., Die Hemmung der Noradrenalin-Aufnahme des Herzens durch Reserpin und die Wirkung von Tyramin. *Arch. exp. Path. Pharmak.* 1960. *240.* 234—241.

MUSCHOLL, E. and M. VOGT, The action of reserpine on the peripheral sympathetic system. *J. Physiol.* (Lond.) 1958. *141.* 132—155.

ROSELL, S. and A. ROSÉN, The functional state of vasomotor nerves to skeletal muscle vessels in reserpinized cats. *Acta physiol. scand.* 1961. *52.* 53—61.

SCHÜMANN, H. J., Über die Verteilung von Noradrenalin und Hydroxytyramin in sympatischen Nerven (Milznerven). *Arch. exp. Path. Pharmak.* 1958. *234.* 17—25.

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Monosynaptically Evoked Inhibitory Post-Synaptic Potentials in Motoneurones

By

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Abstract

Eide, E., A. Lundberg and P. Voorhoeve. *Monosynaptically evoked inhibitory post-synaptic potentials in motoneurones.* Acta physiol. scand. 1961. *53.* 185—195. — Intracellular recording from motoneurones was made in cats with intact dorsal roots and in chronically de-afferented cats. Stimulation of the gray matter was performed with electrodes inserted into the spinal cord. Large excitatory and inhibitory post-synaptic potentials were evoked by these stimuli in alpha motoneurones after a latency of about 0.5 msec. It is concluded that excitatory and inibitory interneurones were activated and that the synaptic delay at inhibitory and excitatory synapses is the same.

The first measurement of synaptic delays in the central nervous system was made by Lorente de Nó (1935), who found a minimal delay of 0.5 msec for transmission of excitation to motoneurones. Later investigations with recording of the onset of the monosynaptic excitatory postsynaptic potential (EPSP) relative to the presynaptic volley have given values of 0.3—0.35 msec (Brock, Coombs and Eccles 1952).

Estimates of the synaptic delay at inhibitory synapses in the central nervous system rest on indirect evidence. The relevant investigations have largely been made on the Ia inhibitory pathway, the start being Lloyd's (1946) description of direct inhibition, *i.e.* the inhibition of monosynaptic reflexes caused by Ia impulses from the antagonist muscle. Lloyd found that this inhibition was discernible when the inhibitory and excitatory volleys arrived approximately simultaneously at the spinal cord and concluded that the inhibitory action was exerted monosynaptically, the inherent assumption being that the

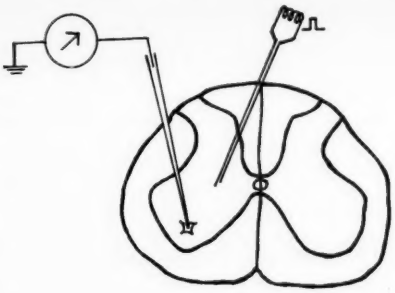

Fig. 1. Drawing of the arrangement of stimulating and recording electrodes.

synaptic delay at inhibitory and excitatory synapses is of the same order. With intracellular recording of the inhibitory post-synaptic potential (IPSP) the central latency for the Ia inhibitory synaptic action could be measured accurately and was found to be 1.3 msec or about 0.8 msec longer than for the monosynaptic Ia EPSP (Brock *et al.* 1952).

In 1956 Eccles, Fatt and Landgren postulated that an interneurone is interpolated in the Ia inhibitory pathway and that the delay at inhibitory synapses would be of the same order as at excitatory or about 0.3 msec. Investigations on other inhibitory pathways from somatic afferents to motoneurones revealed central latencies for the IPSPs that were so long in relation to the assumed synaptic delay of 0.3 msec that one or several synaptic relays were postulated (Eccles *et al.* 1956, Eccles, Eccles and Lundberg 1957 b, Curtis, Krnjević and Miledi 1958, Eccles and Lundberg 1959, Frank and Sprague 1959). The same holds true for the recurrent Renshaw inhibition from motor axon collaterals (Eccles, Fatt and Koketsu 1954) and for the inhibitory action exerted by impulses in descending spinal pathways (Preston and Whitlock 1960). Similar findings have also been made on the cells of the dorsal and ventral spino-cerebellar tracts (Curtis, Eccles and Lundberg 1958, Eccles, Hubbard and Oscarsson 1961, Eccles, Oscarsson and Willis, 1961). Pending confirmation of the basic assumption these findings add support to the generalizing suggestion (Eccles *et al.* 1956, Eccles 1957) that every inhibitory pathway in the central nervous system has a final stage short-axon inhibitory interneurone.

Lloyd and Wilson (1959) have recently claimed that for the Ia pathway the IPSP is not the primary agent of the inhibition but this claim has been effectively refuted by Araki, Eccles and Ito (1960).

In order to obtain further evidence on the synaptic delay at inhibitory synapses it would be desirable to record monosynaptically evoked IPSPs. In the present experiments a stimulus has been applied through electrodes inserted into the gray matter and the latency of IPSPs and EPSPs evoked in motoneurones has been found to be about 0.5 msec. A preliminary report has been given (Eide, Lundberg and Voorhoeve 1960).

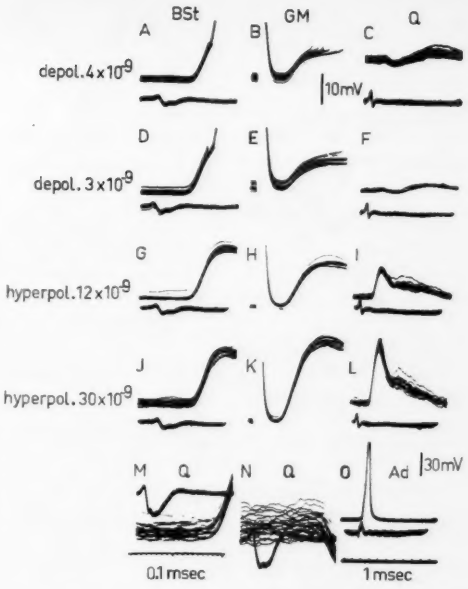

Fig. 2. Intracellular recording with a KCl-electrode from a posterior biceps-semitendinosus (BSt) motoneurone, internal positivity (depolarization) being signalled upwards. Lower traces (except in M) were recorded from the dorsal root entry zone. A, D, G and J in the left column were obtained on stimulation at constant strength of the BSt nerve. In B, E, H and K the gray matter (GM) was stimulated at constant strength through electrodes inserted approximately as shown in Fig. 1. The quadriceps nerve was stimulated at constant strength in C, F, I and L, which were recorded at the slow sweep speed, and in M and N at faster sweep speed. The S1 ventral root was stimulated in O. Current was passed through the recording electrode and for each horizontal row the current flow is indicated in amperes.

Methods

The experiments were made on 5 cats under light anaesthesia from pentobarbitone sodium. In two experiments the dorsal roots were intact and in the remaining three the animals were de-afferented by removal of the spinal ganglia L6—S1 1—3 weeks before the experiment. The spinal cord was transected in L1 and the ipsilateral latero-ventral funicles were dissected for stimulation. In one experiment the dorsal part of the lateral funicle and the ventral quadrant were dissected for separate stimulation. Intracellular recording was made in the L7 and S1 segment as described by BROCK *et al.* (1952). The recording micro-electrode was inserted from the lateral aspect of the cord. The steel electrodes used for stimulation were insulated, except for the tip, and inserted through the dorsal column as shown in Fig. 1. The stimulating electrodes were inserted to a depth of 2.5—3 mm. The distance between the tips of the stimulating electrodes was about 0.5 mm, the ventral electrode being cathode. Square wave pulses with a duration of 0.1 msec were delivered via a radio frequency isolation unit.

All records consist of many superimposed traces.

Results

A comparison of synaptic actions evoked by impulses in primary afferents and by stimulation of the gray matter.

The intracellular records in Fig. 2 are from a posterior biceps-semitendinosus (BSt) motoneurone and show the synaptic potentials evoked on stimulation of the BSt nerve (A, D, G, J), of the gray matter (GM) (B, E, H, K) through steel electrodes inserted as shown in Fig. 1 and of the quadriceps nerve (C,

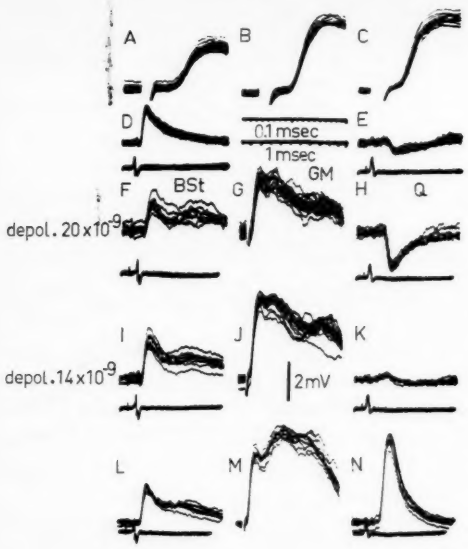

Fig. 3. As in Fig. 2, intracellular recording with a KCl-electrode. Stimulation of the gray matter at increasing strength in A—C and at constant strength in G, J and M. The BSt nerve was stimulated in D, F, I and L and the quadriceps nerve in E, H, K and N. The lower horizontal rows of records were obtained at the indicated current flow through the recording electrode. A—C were obtained at the fast, all the other records at the slow sweep speed.

F, I, L, M, N). The recording micro-electrode was filled with 3 M KCl-solution and in order to analyse inhibitory and excitatory components of the post-synaptic potentials (PSPs) evoked by GM stimulation, current was passed through the recording micro-electrode to change the membrane potential (Coombs, Eccles and Fatt 1955). At a depolarizing current of 4×10^{-9} A, the Ia IPSP from the quadriceps (C) was in the hyperpolarizing direction but when the current was decreased to 3×10^{-9} A, the Ia impulses from quadriceps (F) hardly evoked any change of the membrane potential (*cf.* Coombs *et al.* 1955). Hence the depolarizing effect in E shows the size of the EPSP evoked by the GM stimulus. The latency from the shock artefact to the onset of the EPSP in E is 0.50 msec, as compared with the central latency of 0.55 from the Ia EPSP evoked from the BSt nerve in D (measured from the incoming volley, lower trace, to the onset of the EPSP). When the membrane was hyperpolarized so as to reverse the Ia IPSP from quadriceps (I and L) the depolarizing effect of GM stimulation increases in H and K. This increase in depolarization from E must be due to the superposition of a reversed IPSP. This is proved by the fact that the Ia EPSP from the BSt nerve does not change proportionally in G and J. A comparison of record E and K reveals that the reversed IPSP in K and the EPSP in E have a similar time-course and that the latency of the IPSP is of the same order as of the EPSP. The latency of the depolarization in K is 0.46 msec as compared with 0.50 for the EPSP in E. This difference is, however, hardly significant since the onset is easier

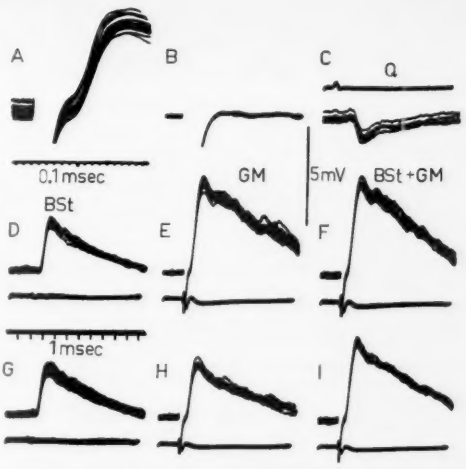

Fig. 4. As in Fig. 2, intracellular recording with a KCl electrode. Stimulation of the gray matter in A, B, E and H. B shows the extracellular field after a slight withdrawal of the recording electrode from the cell. The maximal homonymous EPSP from BSt is shown in D and G. F and I were obtained on stimulation of the BSt nerve and of the gray matter at the same strength that was used to evoke the responses in E and H respectively. In record C the quadriceps nerve was stimulated. This record, obtained at the end of the series, shows that the IPSP was still in the hyperpolarizing direction. A and B were taken at the fast and all the other records at the slow sweep speed.

to measure accurately in K than in E. For comparison the onset of the Ia IPSP from quadriceps is shown at fast sweep speed in M where it is reversed and in N during passage of a depolarizing current (6×10^{-9} A). The central latency is 1.3 msec as found by BROCK *et al.* (1952).

In other BSt cells GM stimulation evoked only a short-latency EPSP with no evidence of an early IPSP. This is illustrated in Fig. 3. Records A—C, at fast sweep speed, show synaptic potentials obtained at increasing strength of GM stimulation. Record E, taken afterwards illustrates that the Ia IPSP from quadriceps had not reversed, hence the synaptic potentials in A—C are EPSPs. In F—N the synaptic potentials, evoked from the BSt nerve (F, I, L), from GM stimulation (G, J, M) and from the quadriceps nerve (H, K, N) are shown during passage of current through the recording micro-electrode. When during hyperpolarization the Ia IPSP reverses (N) this occurs without increase of the short-latency PSP in M, hence there is no evidence that the GM volley in this case evoked any short-latency IPSP, although record M gives evidence of a polysynaptic IPSP.

It would be expected that GM stimulation activates Ia afferents and this is proved by the records in Fig. 4. The Ia EPSP from the BSt nerve is shown in D and the EPSP evoked by strong GM stimulation in E. With combined stimulation of the BSt nerve and the gray matter in F there was hardly any increase from E, which indicates a considerable but not complete occlusion. On the other hand with the weaker GM stimulus in H there is only a moderate occlusion. The size of the EPSP in I is 77 % of the sum of the EPSPs in G and H. Hence in Fig. 4 many of the Ia afferents were activated in the high threshold range of GM stimulation. BSt motoneurones receive Ia EPSPs from gracilis (ECCLES, ECCLES and LUNDBERG 1957 a) but this action is

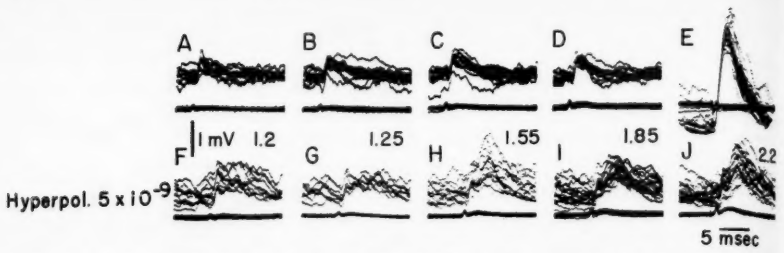

Fig. 5. Intracellular recording with a KCl-electrode from a gastrocnemius-soleus motoneurone. Record E shows the maximal homonymous EPSP from the gastrocnemius-soleus nerve. The ipsilateral dissected latero-ventral funicles were stimulated in A—D and F—J. Corresponding records in A—D and F—I were obtained at the same strength which is indicated, expressed as multiples of the threshold strength. Hyperpolarizing current (5×10^{-9} A) was passed through the micro-electrode in F—J and in E. The distance from the stimulating cathode in L1 to the site of micro-electrode recording in S1 was 90 mm. All the records were obtained at the same amplification.

small and cannot account for the large difference in size between the EPSPs in D and E. There are descending pathways with monosynaptic excitatory action (Fig. 5) but as will be discussed in the next section there is evidence that they were not activated by a stimulus applied as shown in Fig. 1 (see below). It is therefore assumed that excitatory interneurones were stimulated.

Intracellular recording was also used to investigate the effect of volleys descending from the upper lumbar region. Monosynaptic EPSPs were evoked from the ipsilateral ventral quadrants (presumably from the vestibulo-spinal tract) but no short-latency IPSPs were found on stimulation of the ipsilateral or the contralateral latero-ventral funicles. The briefest segmental latency found was 1.3 msec as is illustrated in Fig. 5. A KCl-electrode was used for recording and in A—D only the monosynaptic EPSP appears. Hyperpolarizing current was passed through the electrode in F—J and a second depolarizing wave appears, which must be a reversed IPSP. The onset of this reversed IPSP, which is evoked by activity in low threshold fibres occurs after a latency that is 0.9 msec longer than for the monosynaptic EPSP. Hence it can be concluded that this inhibitory connection is disynaptic. It can further be postulated that the IPSP evoked by stimulation of the gray matter in Fig. 2 is due to stimulation of interneurones and not of long descending pathways with monosynaptic inhibitory action on motoneurones. It is possible that the inhibitory interneurones activated to evoke the IPSP in Fig. 2 belonged to the Ia inhibitory pathway but no attempt was made to prove this. There are other ipsilateral inhibitory pathways from various hind-limb afferents to flexor motoneurones (HAGBARTH 1952, ECCLES and LUNDBERG 1959, HOLMQVIST and LUNDBERG 1959). In addition there must be interneurones mediating crossed inhibitory actions and presumably also interneurones mediating inhibition from descending pathways.

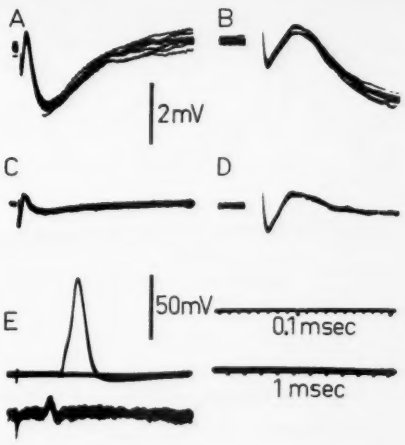

Fig. 6. The experiment was made on a chronically de-afferented cat with intact ventral roots. Recording was made with a K_2SO_4 electrode. The gray matter was stimulated in A—D at a constant strength. A and B were recorded intracellularly and the corresponding records in C and D extracellularly after a slight withdrawal of the recording electrode. The antidromic response in E was evoked from the gastrocnemius-soleus nerve. A, C, E were obtained at the slow, and B, D at the fast sweep speed.

Synaptic actions evoked by stimulation of the gray matter in chronically de-afferented animals

In order to facilitate the analysis experiments were also made on chronically de-afferented animals: after degeneration of the primary afferents, one to three weeks after removal of the ipsilateral spinal ganglia L6—S1. Recordings were made with electrodes filled either with 0.6 M K_2SO_4- or with 3 M KCl-solution. With sulphate electrodes the IPSPs do not reverse; KCl electrodes, on the other hand, have the advantage of lower resistance and also permit analysis of the admixture of composite PSPs by the passage of current.

The records in Fig. 6 were obtained from a gastrocnemius-soleus motoneurone and the slow conduction velocity of its axon (record E) indicates that it belonged to soleus although this was not verified by measurement of its after-potential (*cf.* ECCLES, ECCLES and LUNDBERG 1958). The records show the IPSP evoked by GM stimulation at two sweep speeds in A and B and the corresponding records below are the extracellular fields in response to an identical GM stimulus after withdrawal of the recording electrode. The latency of the IPSP in B is 0.53 msec.

The records in Fig. 7 are also from a gastrocnemius-soleus motoneurone. The recording electrode was filled with 3 M KCl. In order to prevent the IPSPs from reversing a depolarizing current of 10×10^{-9} A was passed through the electrode in A—D. The IPSP evoked by GM stimulation is shown in A at slow and in B at fast sweep speed and should be compared with the extracellular fields in M and N. Records C—D show the Renshaw IPSPs (*cf.* ECCLES *et al.* 1954) evoked from the indicated nerves at this level of depolarization. In E—H obtained without current flow these IPSPs reversed and actually excited the motoneurone. The series I—L in Fig. 7 was obtained

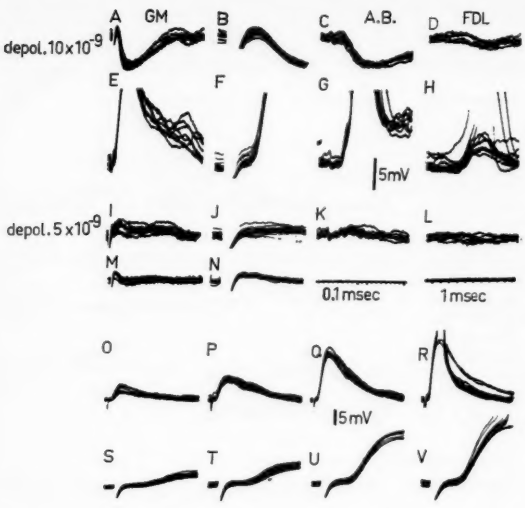

Fig. 7. Recording with a KCl-electrode in a chronically de-afferented cat with intact ventral roots. A—L and O—V are intracellular records from a gastrocnemius-soleus motoneurone; M and N were obtained extracellularly after a slight withdrawal of the electrode. The gray matter was stimulated at constant strength in A, B, E, F, I, J, M and N and at increasing strength in the two lower rows (O—V) where corresponding records were evoked at the same strength but recorded at different sweep speeds. The nerves to anterior biceps (A. B.) and to flexor digitorum longus (FDL) were stimulated in C, G, K and D, H, L respectively. A depolarizing current of the strength indicated was passed through the recording electrode in A—D and I—L. Reversal of the IPSPs in E—H and O—V occurred without any passage of hyperpolarizing current. B, F, J, N and S—V were obtained at the fast, all other records at the slow sweep speed. Observe that records O—V were taken at a lower amplification.

with a depolarizing current of 5×10^{-9} A chosen to maintain the membrane potential at equilibrium level for the Renshaw IPSPs as is illustrated in records K and L. Under these conditions GM stimulation is also ineffective in evoking a synaptic potential and it can therefore be concluded that few or no excitatory interneurones with effects on this cell were activated by the GM stimulus. This conclusion is based on the assumption that the Renshaw IPSP is pure and does not conceal an excitatory effect. Previous investigations have not given evidence of an excitatory admixture in nembutalized spinal cats (Eccles *et al.* 1954) although this occurs in unanaesthetized preparations (Wilson 1959).

In the de-afferented cats successful measurements were made on 14 flexor and 21 extensor motoneurones. The mean latency for EPSPs was 0.54 and for IPSPs 0.52 msec. The small difference is probably entirely explained by the fact that the onset of the IPSP could be more accurately measured since in many of the cells current was passed to reverse the IPSP and with this procedure it was easier to measure the onset. In the majority of motoneurones to extensors the inhibitory effects dominated, whereas in flexor motoneurones excitatory effects were prevalent. The majority of interneurones stimulated are probably concerned with transmission from hindlimb afferents and this distribution may reflect the flexor reflex pattern. However, in a few extensor motoneurones excitation dominated as shown in the gastroc-

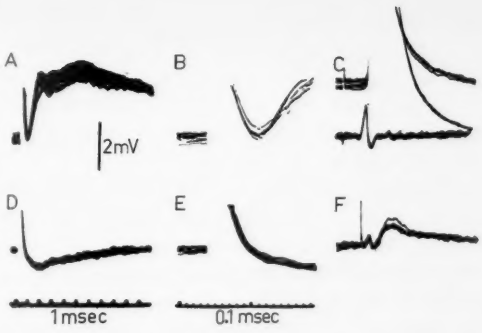

Fig. 8. Recording with a K_2SO_4-electrode in a chronically de-afferented cat. A—C and F are intracellular records from a gastrocnemius-soleus motoneurone. D and E are the extracellular fields obtained after a slight withdrawal of the recording electrode. The gray matter was stimulated at constant strength in A, B, D and E. A and D were recorded at slow, B and E at fast sweep speed. The gastrocnemius-soleus nerve was stimulated in C and the ipsilateral latero-ventral funicles dissected in L2 were stimulated in F.

nemius-soleus motoneurone of Fig. 8: there is a short-latency monosynaptic EPSP (latency in B 0.58 msec) as well as a later polysynaptic EPSP which can be observed at the slow sweep speed in A. Record F shows the maximal monosynaptic EPSP evoked by a descending volley in the ipsilateral latero-ventral funicles (probably in the vestibulo-spinal tract). It is assumed that the EPSPs evoked by GM stimulation are due to activation of interneurones and not of these descending fibres. Our reason for this assumption is that in a number of cells in which GM stimuli failed to evoke EPSPs at a strength slightly submaximal for direct excitation of the motoneurone relatively large monosynaptic EPSPs were nevertheless evoked by the descending volley. A more strict proof could have been provided by experiments on chronic spinal and de-afferented animals, but in this investigation we were concerned mainly with the inhibitory effects.

Discussion

The 'inhibitory interneurone' hypothesis (Eccles *et al.* 1956) was based on experiments with the Ia inhibitory pathway to motoneurones and until now the evidence that an interneurone is interpolated in this path was threefold:

1) Ia volleys from the quadriceps nerve evoke spike potentials in the posterior biceps-semitendinosus nucleus after a central latency of about one msec, thus preceding the onset of the IPSP by 0.3 msec. These spike potentials presumably represent activity in preterminal fibres of the Ia inhibitory interneurones to BSt cells (Eccles *et al.* 1956).

2) The connection of group Ia fibres in the lumbar cord is entirely in agreement with a postulated relay in the intermediate nucleus of Cajal and there is anatomical and physiological evidence that the axons of these cells terminate on motoneurones or in motor nuclei (Szentagothai 1951, Eccles, Fatt, Landgren and Winsbury 1954, Eccles *et al.* 1956, Curtis 1959, *cf.* Eccles 1957, 1961).

3) Spatial facilitation has been demonstrated in the Ia inhibitory pathway (Eccles and Lundberg 1958). This was shown in different ways, the most spectacular being the experiments in which stimulation of any one of 5 branches from the quadriceps nerve failed to evoke a Ia IPSP in BSt motoneurones, whereas a large IPSP could be recorded on simultaneous stimulation of these branches.

Altogether these findings constitute strong circumstantial evidence in favour of the 'inhibitory interneurone' hypothesis but in view of the paramount importance of this hypothesis it was desirable to test it to the utmost. The only possible alternative would be to assume that the delay at inhibitory synapses is longer than at excitatory. The present experiments have excluded this possibility. The onset of the IPSP, evoked by a stimulus applied to the gray matter, occurred after a mean latency of 0.52 msec and a minimal latency of 0.46 msec. The corresponding values for the EPSPs evoked by these stimuli were 0.54 and 0.50 respectively. It has already been pointed out that this difference in latency between the EPSP and IPSP is not significant. It is concluded that the synaptic delay at excitatory and inhibitory synapses is the same.

It was to be expected that the latency for PSPs evoked by electrical stimulation should exceed the one derived by Brooks and Eccles (1947), Brock *et al.* (1952) and Eccles *et al.* (1956). The utilization time for the stimulus and the conduction time to the terminals probably accounts for most of the difference. On the other hand, measurements of the interval between the presynaptic spike and the onset of the PSPs may give values for the synaptic delay on the low side, because the onset of the PSP depends on the fastest fibres, whereas the mean velocity is measured in the presynaptic recording.

There was no indication that fibres in the upper lumbar region have monosynaptic inhibitory connections with motoneurones in L7 and S1 but a disynaptic inhibitory connection was found (Fig. 5) (*cf.* Preston and Whitlock 1960). The monosynaptic IPSPs evoked on stimulation of the gray substance presumably are due to activation of interneurones and the same is postulated for the EPSPs evoked in the chronically de-afferented cats. The stimulation technique could be used to locate excitatory and inhibitory interneurones which terminate on motoneurones.

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References

Araki, T., J. C. Eccles and M. Ito, Correlation of the inhibitory post-synaptic potential of motoneurones with the latency and time course of inhibition of monosynaptic reflexes. J. Physiol. (Lond.) 1960. *154*. 354—377.

Brock, L. G., J. C. Coombs and J. C. Eccles, The recording of potentials from motoneurones with an intracellular electrode. J. Physiol. 1952. (Lond.) *117*. 431—460.

BROOKS, C. McC. and J. C. ECCLES, Electrical investigation of the monosynaptic pathway through the spinal cord. J. Neurophysiol. 1947. *10.* 251—274.

COOMBS, J. S., J. C. ECCLES and P. FATT, The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. J. Physiol. (Lond.) 1955. *130.* 326—373.

CURTIS, D. R., Pharmacological investigations upon inhibition of spinal motoneurones. J. Physiol. (Lond.) 1959. *145.* 175—192.

CURTIS, D. R., J. C. ECCLES and A. LUNDBERG, Intracellular recording from cells in Clarke's column. Acta physiol. scand. 1958. *43.* 303—314.

CURTIS, D. R., K. KRNJEVIĆ and R. MILEDI, Crossed inhibition of sacral motoneurones. J. Neurophysiol. 1958. *21.* 319—326.

ECCLES, J. C., *The physiology of nerve cells.* Baltimore, Johns Hopkins Press. 1957.

ECCLES, J. C., The nature of central inhibition. Proc. roy. Soc. B. 1961. *153.* 445—476.

ECCLES, J. C., R. M. ECCLES and A. LUNDBERG, The convergence of monosynaptic excitatory afferents to many different species of alpha motoneurones. J. Physiol. (Lond.) 1957 a. *137.* 22—50.

ECCLES, J. C., R. M. ECCLES and A. LUNDBERG, Synaptic actions on motoneurones caused by impulses in Golgi tendon organ afferents. J. Physiol. (Lond.) 1957 b. *138.* 227—252.

ECCLES, J. C., R. M. ECCLES and A. LUNDBERG, The action potentials of the alpha motoneurones supplying fast and slow muscles. J. Physiol. (Lond.) 1958. *142.* 275—291.

ECCLES, J. C., P. FATT and K. KOKETSU, Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. J. Physiol. (Lond.) 1954. *126.* 524—562.

ECCLES, J. C., P. FATT and S. LANDGREN, The central pathway for the direct inhibitory action of impulses in the largest afferent nerve fibres to muscle. J. Neurophysiol. 1956. *19.* 75—98.

ECCLES, J. C., P. FATT, S. LANDGREN and G. J. WINSBURY, Spinal cord potentials generated by volley in the large muscle afferents. J. Physiol. (Lond.) 1954. *125.* 590—606.

ECCLES, J. C., J. I. HUBBARD and O. OSCARSSON, Intracellular recording from cells of the ventral spino-cerebellar tract. 1961. In press.

ECCLES, J. C., O. OSCARSSON and W. WILLIS, Synaptic action of group I and II afferent fibres of muscle on the cells of the dorsal spino-cerebellar tract. J. Physiol. (Lond.) 1961. In Press.

ECCLES, R. M. and A. LUNDBERG, The synaptic linkage of 'direct' inhibition. Acta physiol. scand. 1958. *43.* 204—215.

ECCLES, R. M. and A. LUNDBERG, Synaptic actions in motoneurones by afferents which may evoke the flexion reflex. Arch. ital. Biol. 1959. *97.* 199—221.

EIDE, E., A. LUNDBERG and P. VOORHOEVE, The synaptic delay at inhibitory synapses. J. Physiol. (Lond.) 1960. *154.* 30P.

FRANK, K. and J. M. SPRAGUE, Direct contralateral inhibition in the lower sacral spinal cord. Exp. Neurol. 1959. *1.* 28—43.

HAGBARTH, K. E., Excitatory and inhibitory skin areas for flexor and extensor motoneurones. Acta physiol. scand. 1952. *26.* Suppl. 94. 1—58.

HOLMQVIST, B. and A. LUNDBERG, Differential supraspinal control of spinal reflex actions from high-threshold muscle afferents. J. Physiol. (Lond.) 1959. *148.* 70—71P.

LLOYD, D. P. C., Facilitation and inhibition of spinal motoneurones J. Neurophysiol. 1946. *9.* 421—438.

LLOYD, D. P. C. and V. J. WILSON, Functional organization in the terminal segments of the spinal cord with a consideration of central excitatory and inhibitory latencies in monosynaptic reflex systems. J. gen. Physiol. 1959. *42.* 1219—1231.

LORENTE DE NÓ, R., The synaptic delay of motoneurons. Amer. J. Physiol. 1953. *111.* 272—282.

PRESTON, J. B. and D. G. WHITLOCK, Precentral facilitation and inhibition of spinal motoneurones. J. Neurophysiol. 1960. *23.* 154—170.

SZENTAGOTHAI-SCHIMERT, J., Short propriospinal neurones and intrinsic connections of the spinal gray matter. Acta morph. Hung. 1951. *1.* 81—94.

WILSON, V. J., Recurrent facilitation of spinal reflexes. J. gen. Physiol. 1959. *42.* 703—713.

Acta physiol. scand. 1961. 53. 196.

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Uptake of Catecholamines by Adrenergic Nerve Granules

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Nerve granules were prepared from bovine splenic nerves (Euler and Lishajko 1961) and incubated at room temperature and pH 7.0 in isotonic potassium phosphate to which catecholamines had been added in different concentrations. In the presence of 10 μg noradrenaline (NA) per ml in the incubation fluid no spontaneous loss of the granule-bound NA was noted in 60 min at 20° C as against a loss of 67 per cent when the NA concentration was 1 μg per ml or less in the incubation fluid. No loss was observed after incubation even for 120 min in NA 20 μg/ml; with 5 μg/ml NA the loss was 50 per cent during the same time.

After previous incubation of the granules for 120 min, causing a depletion of about 80 per cent, and subsequent incubation for 60 min with NA after sedimentation and resuspension of the granules, an uptake of NA was observed. This was small but measurable with 5 μg/ml NA in the incubation fluid and increased with raising concentrations of NA. With 20 μg/ml NA in the incubation fluid the granules were repleted up to 95 per cent of the original amount whereas the controls had lost over 80 per cent of their NA. Reserpine 10 μg/ml did not prevent the uptake of NA by depleted granules. Adrenaline was taken up by nerve granules in similar amounts as NA.

The results show that isolated nerve granules can take up catecholamines which has previously been shown to occur for dopamine in adrenal medullary granules (Bertler et al. 1961). Assuming similar conditions in vivo as in vitro, it might be hypothesized that while NA is retained in the storage granules when the concentration in the axoplasm is about 10 μg/ml, as actually found in vivo, it may be released from the granules at lower extragranular concentrations following NA flux through the axon membrane during nerve stimulation.

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References

Bertler, A., G. Hall, N.-Å. Hillarp and E. Rosengren, Uptake of dopamine by the storage granules of the adrenal medulla in vitro. *Acta physiol. scand.* 1961. *52.* 167—170.

Euler, U. S. v. and F. Lishajko, Noradrenaline release from isolated nerve granules. *Acta physiol. scand.* 1961. *51.* 193—203.

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